WAVE Bioreactor Systems Cell culture procedures

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WAVE Bioreactor Systems

Cell culture procedures

Several protocols have been developed for cultivating various cell types in the WAVE Bioreactor™ system. These are provided as a useful starting point for your own experiments. We are constantly developing and updating procedures based on user comments and experience. Please visit www.gelifesciences.com/wave for the latest information.

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Cultivation of cells on Hillex microcarriers using WAVE Bioreactor Systems 2/10 and 20/50

Application

Microcarriers are very small beads designed to provide a substrate for cells to grow on while in suspension. Because WAVE Bioreactor™ systems produce a very low-shear environment while maintaining mixing and oxygenation, they are ideal for the use of microcarriers. Furthermore, the volume in Cellbag™ disposable bioreactors can be increased by a factor of 10. This allows microcarrier cultures to be started at very small volumes with high densities of both carriers and cells for better cell-to-microcarrier contact. Media can then be added to bring the culture to final volume.

This protocol refers to the use of microcarriers made by SoloHill Engineering, Inc. (Ann Arbor, MI). Hillex™ microcarriers are polystyrene beads treated with a variety of surface coatings designed for different cell lines. Contact the manufacturer to determine the best surface treatment for a particular cell line.

Inoculation

Inflate a Cellbag bioreactor with air and 10% CO_2 until it is rigid, add media and clamp the inlet and outlet filters. Start rocking at 15 rocks per minute (rpm) and an angle of 7°. Allow the temperature and pH to equilibrate. The initial volume should be about 50% of the final culture volume. Follow the manufacturer's instructions on preparing the microcarriers. Allow the pH to equilibrate. Hillex microcarriers can absorb phenol red, which will create the impression that the pH has changed. Monitor the pH by other methods. Follow the manufacturer's recommendations for the amount of microcarriers to use and add the entire amount to the Cellbag bioreactor. The oxygenation capability of the WAVE Bioreactor system means it is generally possible to use a large amount of microcarriers. Add the cell suspension to the Cellbag bioreactor. Generally, an initial cell density of 0.1 to 0.5 × 10^6 cells/mL is desirable. Lower cell densities can be used depending on the cell line. Keep the inlet and outlet filter clamped and begin rocking the Cellbag bioreactor at a speed of 20 rpm and an angle of 7°. Do not rock too slowly. It is important to get good cell-to-microcarrier contact allowing the cells to stick to the microcarriers and begin growth. The attachment process can take several hours or overnight. For cells that have difficulty attaching it might be necessary to try intermittent rocking. Rock the Cellbag bioreactor for a minute or two then stop for 20 min. Continue until the cells have attached.

Operation

Once the cells have attached, add the remaining amount of media to bring the culture up to final volume. Monitor cell density, viability, and metabolism while the cells are growing. Due to the small opening in the Clave™ sample port, larger microcarrier/cell complexes might not be pulled from the Cellbag bioreactor intact. In those cases, use one of the other tubing connections to remove a sample. Keep the bioreactor rigidly inflated at all times. The oxygen levels of the culture are critical to successful cell growth and production. Monitor the oxygen levels with a dissolved oxygen (DOOPT) system, WAVEPOD™ Controller, or offline blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture. It is best to maintain a low rpm and angle while maintaining sufficient oxygen and keeping the microcarriers/cells suspended.

As the cells continue to grow, the media will eventually become spent. Media exchange can be easily accomplished by shutting off the rocking. With the platform tipped forward, the microcarrier/cell complexes will settle to the bottom edge of the Cellbag bioreactor within minutes. The media can then be pumped out without removing any of the microcarriers. Up to 90% of the culture volume can be removed in this manner. Add fresh prewarmed media and resume rocking at the previous settings. Avoid allowing the cells to sit for extended periods otherwise oxygen depletion can become a problem.

Culture parameters

Rocking speed

The rocking speed is dependent on the culture volume, cell density, and Cellbag biopreactor size. For Cellbag-2L, -10L, and -20L, set the speed at 15 to 20 rpm initially. For very low cell densities, an initial speed of 15 rpm is sufficient. Generally, increase the rpm as the oxygen demand increases. It is important to maintain sufficient rocking

speed to keep the microcarriers in suspension. Increase the speed to 20 to 25 rpm as the cell density increases. When at high cell density, the speed might need to be as high as 25 rpm. For Cellbag-50L, set the speed at 12 to 18 rpm initially. At low cell density, set the speed at 12 rpm. When at maximum density, use a speed of 22 rpm. These are general guidelines only. Do not allow the microcarriers to settle to the bottom of the bioreactor. Monitor the oxygen levels and adjust the rpm as needed.

Rocking angle

For Cellbag-2L, -10L, and -20L, an initial angle of 6° is sufficient. For Cellbag-50L, use an initial angle of 5°. When the bioreactor is at maximum cell density, an angle of 7° or 8° might be needed when using Cellbag-2L, -10L, or -20L. For Cellbag-50L, an angle of 6° to 7° can be used when at maximum density. Increase the rocking angle and decrease speed if excessive foaming is observed. Experience shows that this will help to break up the foam. Reduce the angle if cells begin to fall off the microcarriers. It is important to monitor the oxygen levels of the culture and adjust the rocking angle as needed.

Aeration rate

The Cellbag bioreactor should be kept rigidly inflated. During bioreactor inflation, a flow rate of up to 0.5 liters per minute (Lpm) can be used. Keep the filters clamped during the initial stages of the culture. Once vigorous growth is observed, set the flow rate to 0.1 Lpm for the Cellbag-2L, 0.2 Lpm for Cellbag-10L and Cellbag-20L, and 0.3 Lpm for Cellbag-50L during the run.

Temperature

The typical operating temperature for mammalian cells is 36°C to 37°C.

pH control

pH control is critical. Due to the high gas transfer capacity of the WAVE Bioreactor system, pH can drift rapidly. Use the following procedure:

1. Initially inflate the Cellbag bioreactor with 10% CO_2/air. After inflation, add media and microcarriers to the bioreactor and close off the inlet and outlet air filters. Rock at 15 rpm for 1 to 2 h to allow the pH and temperature to completely equilibrate. Before inoculation, check the pH by taking a sample. Adjust if necessary.
2. Inoculate the microcarriers with cells. Leave the inlet and outlet filters closed.
3. Monitor pH, glucose concentration, and cell density. Once the pH and glucose levels start dropping, switch to 5% CO_2/air with continuous airflow through the headspace. This should occur within 24 to 60 h. Once vigorous cell growth occurs, the media pH will not drift upwards and CO_2 concentration in the sweep gas can be used to control pH.
4. Increase the rocking speed and angle to maintain oxygen concentration. Use offline sampling, a DOOPT system, or WAVEPOD Controller to estimate dissolved oxygen concentration.
5. Care should be taken when replacing spent media. Monitor the pH and adjust the CO_2 concentration as cells become acclimated to the fresh media.

Scale up

A typical scale up for a cell line on Hillex microcarriers in a Cellbag-20L is given below. Keep in mind that this is a general guide only. Different cell lines behave differently.

1. Fill 5 L of media into a Cellbag-20L. Add 200 g of Hillex microcarriers and allow the pH to equilibrate. Add enough cell inoculum to give a starting cell count of at least 0.2×10^6 cells/mL. Set the rocking speed to 25 rpm and the angle at 6° overnight. Keep the system at the operating temperature.
2. The next day add 5 L of media. Adjust the speed to 18 rpm and the angle to 6°.
3. Continue the culture for another day until the pH begins to drop. Unclamp the inlet and outlet filters and begin continuous air/CO_2 flow. Monitor the oxygen levels in the culture carefully. Adjust the speed to 20 rpm.
4. Continue the culture for a few more days until glucose levels and low pH indicate the media is spent. Exchange 50% of the media. Monitor pH carefully after the media exchange. Increase the rocking speed to 22 rpm and the angle to 7°.
5. Continue to exchange 50% of the media every second day.

Adjust the volume proportionally for other size Cellbag bioreactors. A Cellbag-10L would start at 2.5 L and Cellbag-2L at 500 mL. When using a Cellbag-50L with a working volume of 25 L, generally use a slightly reduced rpm and angle compared to the settings used for smaller Cellbag bioreactors. For example, use an initial speed of 15 rpm and angle of 5° with a Cellbag-50L rather than the settings given above.

Comments

Excessive rpm and angle can knock cells off the microcarriers. It is important to monitor the oxygen levels in the culture and use the minimum rpm and angle possible while maintaining the appropriate oxygen amounts and keeping the microcarriers suspended.

If pH control becomes a problem after media exchanges, try exchanging 25% of the culture volume every day.

For contact information for your local office, please visit, www.gelifesciences.com/contact

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Cultivation of cells on Cultispher microcarriers using WAVE Bioreactor Systems 2/10 and 20/50

Application

Microcarriers are very small beads designed to provide a substrate for cells to grow on while in suspension. Since the WAVE Bioreactor™ systems produce a very low-shear environment while maintaining excellent mixing and oxygenation, they are ideal for the use of microcarriers. Furthermore, the volume in Cellbag™ disposable bioreactors can be increased by a factor of 10. This allows microcarrier cultures to be started at very small volumes with high densities of both carriers and cells for better cell-to-microcarrier contact. Media can then be added to bring the culture to final volume.

This procedure refers to the use of Cultispher™ microcarriers. Cultispher microcarriers are macroporous cross-linked porcine gelatin beads. They can be dissolved with trypsin allowing nearly 100% of the cells to be recovered.

Inoculation

Inflate a Cellbag bioreactor with air and 10% CO_2 until it is rigid, add media and clamp the inlet and outlet filters. Start rocking at 15 rocks per minute (rpm) and an angle of 7°. Allow the temperature and pH to equilibrate. The initial volume should be about 50% of the final culture volume. Follow the manufacturer's instructions on preparing the microcarriers. Allow the pH to equilibrate. Follow the manufacturer's recommendations for the amount of microcarriers to use and add the entire amount to the Cellbag bioreactor. The oxygenation capability of the WAVE Bioreactor system means it is generally possible to use a large amount of microcarriers. Add the cell suspension to the Cellbag bioreactor. Generally, an initial cell density of 0.1 to 0.5 × 10^6 cells/mL is desirable. Lower cell densities can be used depending on the cell line. Keep the inlet and outlet filter clamped and begin rocking at a speed of 25 rpm and an angle of 6°. Do not rock too slowly. It is important to get good cell-to-microcarrier contact to allow the cells to stick to the microcarriers and begin growth. The attachment process can take several hours or overnight. For cells that have difficulty attaching, it might be necessary to try intermittent rocking. Rock the Cellbag bioreactor for a minute or two then stop for 20 min. Continue until the cells have attached.

Operation

Once the cells have attached, add the remaining amount of media to bring the culture up to final volume. Monitor cell density, viability, and metabolism while the cells are growing. Due to the small opening in the Clave™ sample port, larger microcarrier/cell complexes might not be pulled from the Cellbag bioreactor intact. In those cases, use one of the other tubing connections to remove a sample. Keep the Cellbag bioreactor rigidly inflated at all times. The oxygen levels of the culture are critical to successful cell growth and production. Monitor the oxygen levels with a dissolved oxygen (DOOPT) system, WAVEPOD™ Controller, or offline blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture. It is best to maintain a low rpm and angle while maintaining sufficient oxygen and keeping the microcarriers/cells suspended.

As the cells continue to grow, the media will eventually become spent. Media exchange can be easily accomplished by shutting off the rocking. With the platform tipped forward, the microcarrier/cell complexes will settle to the bottom edge of the Cellbag bioreactor within minutes. The media can then be pumped out without removing any of the microcarriers. Up to 90% of the culture volume can be removed in this manner. Add fresh prewarmed media and resume rocking at the previous settings. Avoid allowing the cells to sit for extended periods otherwise oxygen depletion can become a problem.

Because Cultispher microcarriers are macroporous, it can be difficult to determine the number of cell on the microcarriers. It is best to dissolve the microcarriers in trypsin and count the recovered cells.

Culture parameters

Rocking speed

The rocking speed is dependent on the culture volume, cell density, and Cellbag bioreactor size. For Cellbag-2L, -10L, and -20L set the speed at 15 to 20 rpm initially. For very low cell densities, an initial speed of 15 rpm is sufficient. Generally, increase the rpm as the oxygen demand increases. It is important to maintain enough speed to keep the microcarriers in suspension. Increase the speed to 20 to 25 rpm as the cell density increases. When at high cell density, it might need to be as high as 25 rpm. For

Cellbag-50L, set the speed at 12 to 18 rpm initially. At low cell density, set the speed at 12 rpm. When at maximum density, use a speed of 22 rpm. These are general guidelines only. Do not allow the microcarriers to settle to the bottom of the Cellbag bioreactor. Monitor the oxygen levels and adjust the rpm as needed.

Rocking angle

For Cellbag-2L, -10L and -20L, an initial angle of 6° is sufficient. For Cellbag-50L, use an initial angle of 5°. When the Cellbag bioreactor is at maximum cell density, an angle of 7° or 8° might be needed when using Cellbag-2L, -10L, or -20L. For Cellbag-50L, an angle of 6° to 7° may be used when at maximum density. Increase the rocking angle and decrease speed if excessive foaming is observed. Experience shows that this will help to break up the foam. Reduce the angle if cells begin to fall off the microcarriers. It is important to monitor the oxygen levels of the culture and adjust the rocking angle as needed.

Aeration rate

The Cellbag bioreactor should be kept rigidly inflated. During inflation, a flow rate of up to 0.5 liters per minute (Lpm) can be used. During the initial stages of the culture, keep the filters clamped. Once vigorous growth is observed, set the flow rate to 0.1 Lpm for the Cellbag-2L, 0.2 Lpm for Cellbag-10L and Cellbag-20L, and 0.3 Lpm for Cellbag-50L during the run.

Temperature

Typical operating temperature for mammalian cells is 36°C to 37°C.

pH control

pH control is critical. Due to the high gas transfer capacity of the WAVE Bioreactor system, pH can drift rapidly. Use the following procedure:

1. Initially inflate the Cellbag bioreactor with 10% CO_2/air. After inflation, add media and microcarriers to the bioreactor and close off the inlet and outlet air filters. Rock at 15 rpm for 1 to 2 h to allow the pH and temperature to completely equilibrate. Before inoculation, check the pH by taking a sample. Adjust if necessary.
2. Inoculate the microcarriers with cells. Leave the inlet and outlet filters closed.
3. Monitor pH, glucose concentration, and cell density. Once the pH and glucose levels start dropping, switch to 5% CO_2/air with continuous airflow through the headspace. This should occur within 24 to 60 h. Once vigorous cell growth occurs, the media pH will not drift upwards and CO_2 concentration in the sweep gas can be used to control pH.
4. Increase the rocking speed and angle to maintain oxygen concentration. Use offline sampling, a DOOPT system, or WAVEPOD Controller to estimate dissolved oxygen concentration.
5. Care should be taken when replacing spent media. Monitor the pH and adjust the CO_2 concentration as cells become acclimated to the fresh media.

Scale up

A typical scale up for a cell line on Cultispher microcarriers in a Cellbag-20L is given below. Keep in mind that this is a general guide only. Different cell lines behave differently.

1. Fill 5 L of media into a Cellbag-20L. Add 20 g of Cultispher microcarriers and allow the pH to equilibrate. Add enough cell inoculum to give a starting cell count of at least 0.2×10^6 cells/mL. Set the rocking speed to 25 rpm and the angle at 6° overnight. Keep the system at the operating temperature.
2. The next day, add 5 L of media. Adjust the speed to 18 rpm and the angle to 6°.
3. Continue the culture for another day until the pH begins to drop. Unclamp the inlet and outlet filters and begin continuous air/CO_2 flow. Monitor the oxygen levels in the culture carefully. Adjust the speed to 20 rpm.
4. Continue the culture for a few more days until glucose levels and low pH indicate the media is spent. Exchange 50% of the media. Monitor pH carefully after the media exchange. Increase the speed to 22 rpm and the angle to 7°.
5. Continue to exchange 50% of the media every second day.

Adjust the volume proportionally for other size Cellbag bioreactors. A Cellbag-10L would start at 2.5 L and a Cellbag-2L at 500 mL. When using a Cellbag-50L with a working volume of 25 L, generally use a slightly reduced rpm and angle compared to the settings used for smaller Cellbag bioreactors. For example, use an initial speed of 15 rpm and an angle of 5° with a Cellbag-50L rather then the settings given above.

Comments

Excessive rpm and angle can knock the cells off of the microcarriers. It is important to monitor the oxygen levels in the culture and use the minimum rpm and angle possible while maintaining the appropriate oxygen amounts and keeping the microcarriers suspended.

If pH control becomes a problem after media exchanges, try exchanging 25% of the culture volume every day.

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Cultivation of cells on Cytodex 3 microcarriers using WAVE Bioreactor Systems 2/10 and 20/50

Application

Microcarriers are very small beads designed to provide a substrate for cells to grow on while in suspension. Since the WAVE Bioreactor™ systems produce a very low-shear environment while maintaining excellent mixing and oxygenation, they are ideal for the use of microcarriers. Furthermore, the volume in Cellbag™ disposable bioreactors can be increased by a factor of 10. This allows microcarrier cultures to be started at very small volumes with high densities of both carriers and cells for better cell-to-microcarrier contact. Media can then be added to bring the culture to final volume.

This procedure refers to the use of Cytodex™3 microcarriers. Cytodex 3 microcarriers consist of a thin layer of denatured collagen chemically coupled to a matrix of cross-linked dextran. The denatured collagen layer is susceptible to digestion by a variety of proteases, including trypsin and collagenase. This makes Cytodex 3 a desirable carrier when the cell is the product, or needs to be harvested intact.

Inoculation

Inflate a Cellbag bioreactor with air and 10% CO_2 until it is rigid, add media and clamp the inlet and outlet filters. Start rocking at 15 rocks per minute (rpm) and an angle of 7°. Allow the temperature and pH to equilibrate. The initial volume, including microcarriers, should be about 30% of the final culture volume. Follow the manufacturer's instructions on the amount and preparation of the microcarriers and add the entire amount to the Cellbag bioreactor. Allow the pH to equilibrate. Because of the WAVE Bioreactor system's excellent oxygenation capability, it is generally possible to use a large amount of microcarriers. Add the cell suspension to the Cellbag bioreactor. Generally, an initial cell density of ten cells per microcarrier is desirable. Lower cell densities can be used depending on the cell line. Keep the inlet and outlet filter clamped and begin rocking at a speed of 15 rpm and an angle of 5° for 1 min, every 30 min. During the intervening time, stop the rocker level with heating. Intermittent rocking as described will maximize cell-to-bead contact by allowing for periodic suspension and mixing of all the particles. Intermittent rocking is preferable to continuous slow rocking because slow rocking reduces cell-to-bead contact. Continue this intermittent rocking for 3 h. After the third hour, begin constant rocking at a speed of 6 rpm and an angle of 2°, and begin pumping in medium to achieve final volume over 12 h.

Operation

Once final volume is achieved, increase the rocking speed to 10 rpm and angle to 5°. Monitor cell density, viability, and metabolism while the cells are growing. Due to the small opening in the Clave™ sample port, larger microcarrier/cell complexes might not be pulled from the Cellbag intact. In those cases, use one of the other tubing connections to remove a sample. Keep the Cellbag bioreactor rigidly inflated at all times. The oxygen levels of the culture are critical to successful cell growth and production. Monitor the oxygen levels with a dissolved oxygen (DOOPT) system, WAVEPOD™ Controller, or offline blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture. It is best to maintain a low rpm and angle while maintaining sufficient oxygen and keeping the microcarriers/cells suspended.

As the cells continue to grow, the media will eventually become spent. Media exchange can be easily accomplished by shutting off the rocking. With the platform tipped forward, the microcarrier/cell complexes will settle to the bottom edge of the Cellbag bioreactor within minutes. The media can then be pumped out without removing any of the microcarriers. Up to 90% of the culture volume can be removed in this manner. Add fresh prewarmed media and resume rocking at the previous settings. Avoid allowing the cells to sit for extended periods otherwise oxygen depletion can become a problem.

Culture parameters

Rocking speed

The rocking speed is dependent on the culture volume, cell density, and Cellbag bioreactor size. For Cellbag-2L, -10L, and -20L set at 10 to 15 rpm initially. For very low cell densities, an initial speed of 10 rpm is sufficient. Generally, increase the rpm as the oxygen demand increases. It is important to maintain sufficient rocking speed to keep the microcarriers in suspension. Increase the speed to 15 to 20 rpm as the cell density increases. When at high cell density, the speed might need to be as high as 25 rpm. These are general guidelines only. Do not allow the microcarriers to settle to the bottom of the Cellbag bioreactor. Monitor the oxygen levels and adjust the rpm as needed.

Rocking angle

For Cellbag-2L, -10L, and -20L an initial angle of 5° is sufficient. Increase the rocking angle and decrease speed if excessive foaming is observed. Experience shows that this will help to break up the foam. Reduce the angle if cells begin to fall off the microcarriers. It is important to monitor the oxygen levels of the culture and adjust the rocking angle as needed.

Aeration rate

The Cellbag bioreactor should be kept rigidly inflated. During inflation, a flow rate of up to 0.5 liters per minute (Lpm) can be used. Keep the filters clamped during the initial stages of the culture. Once vigorous growth is observed, set the flow rate to 0.1 Lpm for the Cellbag-2L, 0.2 Lpm for Cellbag-10L and Cellbag-20L, and 0.3 Lpm for Cellbag-50L during the run.

Temperature

Typical operating temperature for mammalian cells is 36°C to 37°C.

pH control

pH control is critical. Due to the high gas transfer capacity of the WAVE Bioreactor system, pH can drift rapidly. Use the following procedure:

1. Initially, inflate the Cellbag bioreactor with 10% CO_2/air. After inflation, add media and microcarriers to the bioreactor and close off the inlet and outlet air filters. Rock at 15 rpm for 1 to 2 h to allow the pH and temperature to completely equilibrate. Before inoculation, check the pH by taking a sample. Adjust if necessary.
2. Inoculate the microcarriers with cells. Leave the inlet and outlet filters closed.
3. Monitor pH, glucose concentration, and cell density. Once the pH and glucose levels start dropping, switch to 5% CO_2/air with continuous airflow through the headspace. This should occur within 24 to 60 h. Once vigorous cell growth occurs, the media pH will not drift upwards and CO_2 concentration in the sweep gas can be used to control pH.
4. Increase the rocking speed and angle to maintain oxygen concentration. Use offline sampling, a dissolved oxygen (DOOPT) system, or WAVEPOD Controller to estimate dissolved oxygen concentration.
5. Care should be taken when replacing spent media. Monitor the pH and adjust the CO_2 concentration as cells become acclimated to the fresh media.

Comments

Excessive rpm and angle can knock cells off the microcarriers. It is important to monitor the oxygen levels in the culture and use the minimum rpm and angle possible while maintaining the appropriate oxygen amounts and keeping the microcarriers suspended. If pH control becomes a problem after media exchanges, try exchanging 25% of the culture volume every day.

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Cultivation of cells on Fibra-Cel carriers using WAVE Bioreactor Systems 2/10 and 20/50

Application

Fibra-Cel™ carriers are small discs about 1 cm in diameter. They are made of polyester fibers on a polyethylene mesh and provide a matrix on which many adherent-dependent cell lines can grow successfully. Cell adhesion to Fibra-Cel carriers is excellent because the cells tend to become embedded in the fibers. Since the cells will grow in the interior of the disks as well as the outside, the available surface area for growth is quite large. The WAVE Bioreactor™ systems produce a very low-shear environment while maintaining excellent mixing and oxygenation, therefore they are ideal for the use with Fibra-Cel carriers.

Due to the their size, Fibra-Cel carriers cannot be pumped into a Cellbag™ disposable bioreactor for use. However, it is possible to introduce the carriers though an optional screw cap port. Alternatively, we can supply Cellbag bioreactors with Fibra-Cel carriers pre-installed and sterile for immediate use.

Inoculation

Aseptically insert the Fibra-Cel carriers through the screw cap port on the Cellbag bioreactor or use a Cellbag bioreactor prefilled with Fibra-Cel carriers. Inflate the Cellbag bioreactor with air and 10% CO_2 until it is rigid, add media, and clamp the inlet and outlet filters. Media volume should be about 50% of the final volume. Start rocking at 15 rocks per minute (rpm) and an angle of 7°. Allow the temperature and pH to equilibrate. Add the cell suspension to the bioreactor. Generally, an initial cell density of 0.5×10^6 cells/mL is desirable. Lower cell densities can be used depending on the cell line. Keep the inlet and outlet filter clamped and begin rocking at a speed of 23 rpm and an angle of 7°. After approximately 30 min, the media should look clear. This is an indication that the cells have become entrapped in the fibers of the disks and are no longer suspended in the media. Reduce the rocking speed and angle to the settings described in "Culture parameters."

Operation

Monitor cell density, viability, and metabolism while the cells are growing. As the cell density increases, add media until the final volume is reached. Since Fibra-Cel carriers are so large, it is not possible to sample the disks directly. Measurements of cell growth should be done indirectly by monitoring cell metabolism. Cell metabolism can be inferred by measuring nutrient levels such as glucose or glutamine, or waste product amounts such as lactic acid and pH. The oxygen levels of the culture are critical to successful cell growth and production. Monitor the oxygen levels with a dissolved oxygen (DOOPT) system, WAVEPOD™ Controller, or offline blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture. It is best to maintain a low rpm and angle while maintaining sufficient oxygen and keeping the Fibra-Cel carriers suspended. As the cells continue to grow, the media will eventually become spent. Media exchange can be easily accomplished by shutting off the rocking. With the platform tipped forward, the Fibra-Cel carriers will settle to the bottom edge of the Cellbag bioreactor within minutes. The media can then be pumped out without removing any of the microcarriers. Up to 90% of the culture volume can be removed in this manner. Add fresh prewarmed media and resume rocking at the previous settings. Avoid allowing the Fibra-Cel carriers to sit for extended periods otherwise oxygen depletion can become a problem.

Culture parameters

Rocking speed

The rocking speed is dependent on the culture volume, cell density, and Cellbag bioreactor size. For Cellbag-2L, -10L, and -20L set at 20 to 23 rpm initially. For very low cell densities, an initial speed of 20 rpm is sufficient. It is important to maintain enough speed to keep the Fibra-Cell carriers in suspension. Increase the speed to 25 to 28 rpm as the cell density increases. For Cellbag-50L, set the speed at 18 to 20 rpm initially. At low cell density, set the speed at 18 rpm. When at maximum density, use a speed of 22 to 25 rpm. These are general guidelines only. Do not allow the Fibra-Cel carriers to settle to the bottom of the Cellbag bioreactor. Monitor the oxygen levels and adjust the rpm as needed.

Rocking angle

For Cellbag-2L, -10L, and -20L an initial angle of 6° is sufficient. For Cellbag-50L, use an initial angle of 5°. When the Cellbag bioreactor is at maximum cell density, an angle of 7° or 8° might be needed when using Cellbag-2L, -10L, and -20L. For Cellbag-50L, an angle of 6° to 7° can be used when at maximum density. Increase the rocking angle and decrease speed if excessive foaming is observed. Experience shows that this will help to break up the foam. Reduce the

angle if the cells begin to fall off the Fibra-Cel carriers. It is important to monitor the oxygen levels of the culture and adjust the rocking angle as needed.

Aeration rate

The Cellbag bioreactor should be kept rigidly inflated. During inflation, a flow rate of up to 0.5 liters per minute (Lpm) can be used. Keep the filters clamped during the initial stages of the culture. Once vigorous growth is observed, set the flow rate to 0.1 Lpm for the Cellbag-2L, 0.2 Lpm for Cellbag-10L and Cellbag-20L, and 0.3 Lpm for Cellbag-50L during the run.

Temperature

Typical operating temperature for mammalian cells is 36°C to 37°C.

pH control

pH control is critical. Due to the high gas transfer capacity of the WAVE Bioreactor system, pH can drift rapidly. Use the following procedure:

1. Initially, inflate the Cellbag bioreactor with 10% CO_2/air. After inflation, add media to the bioreactor and close off the inlet and outlet air filters. Rock at 15 rpm for 1 to 2 h to allow the pH and temperature to completely equilibrate. Before inoculation, check the pH by taking a sample. Adjust if necessary.
2. Inoculate the Fibra-Cel carriers with cells. Leave the inlet and outlet filters closed.
3. Monitor pH, glucose concentration, and cell density. Once the pH and glucose levels start dropping, switch to 5% CO_2/air with continuous airflow through the headspace. This should occur within 24 to 60 h. Once vigorous cell growth occurs, the media pH will not drift upwards and CO_2 concentration in the sweep gas can be used to control pH.
4. Increase the rocking speed and angle to maintain oxygen concentration. Use offline sampling, a DOOPT system, or WAVEPOD™ Controller to estimate dissolved oxygen concentration.
5. Care should be taken when replacing spent media. Monitor the pH and adjust the CO_2 concentration as cells become acclimated to the fresh media.

Scale up

A typical scale up for a cell line on Fibra-Cel carriers in a Cellbag-20L is given below. Keep in mind that this is a general guide only. Different cell lines behave differently.

1. Add the appropriate number of grams of sterile Fibra-Cel carriers into a Cellbag-20L to achieve the desired surface area. Add 5 L of media and allow the pH to equilibrate. Add enough cell inoculum to give a starting cell count of at least 0.5×10^6 cells/mL. Set the rocking speed to 23 rpm and the angle at 7° for 30 min. Keep the system at the operating temperature.
2. Adjust the speed to 20 rpm and the angle to 6°.
3. Continue the culture for another 2 days until the pH begins to drop. Add 2 L of fresh media.
4. The next day, add 3 L of additional media. Unclamp the inlet and outlet filters and begin continuous air/CO_2 flow. Monitor the oxygen levels in the culture carefully. Increase the speed to 22 rpm.
5. Continue the culture for a few more days until glucose levels and low pH indicate the media is spent. Exchange 50% of the media. Monitor pH carefully after the media exchange. Increase the speed to 25 rpm and the angle to 7°.
6. Continue to exchange the 50% of the media every second day. Monitor the oxygen levels and increase the angle and rpm of required.

Adjust the volume proportionally for other size Cellbag bioreactors. A Cellbag-10L would start at 2.5 L and Cellbag-2L at 500 mL. When using a Cellbag-50L with a working volume of 25 L, generally use a slightly reduced rpm and angle compared to the settings used for smaller Cellbag bioreactors. For example, use an initial speed of 18 rpm and angle of 5° with a Cellbag-50L rather then the settings given above.

Comments

It is important to monitor the oxygen levels in the culture and use the minimum rpm and angle possible while at the same time maintaining the appropriate oxygen amounts and keeping the Fibra-Cells suspended.

If pH control becomes a problem after media exchanges, try exchanging 25% of the culture volume every day.

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Cellbag disposable bioreactor pretreatment for the use of chemically defined lipid supplements

Application

Serum-free media and animal serum substitutes reduce the dependence of mammalian and insect cell culture on animal sourced components. Among the substitute products available are chemically defined lipid supplements that allow lipid-dependent cell lines to grow in serum-free media. Some of these supplements can interact with plastic media storage bags such as Cellbag™ disposable bioreactors resulting in the loss of available lipid in the media. This procedure describes a method of pretreating a Cellbag bioreactor to allow the use of chemically-defined lipid supplements. It is important to test the lipid supplement in a Cellbag bioreactor before additional scale up work is performed.

Protocol

1. Install the Cellbag bioreactor on a WAVE Bioreactor™ system rocking platform and inflate normally. Aseptically, add sterile phosphate-buffered saline (PBS) to the final working volume.
2. Aseptically, add enough of the lipid supplement to result in a 5 × concentration of lipid in the PBS.
3. Set the rocks per minute (rpm) and the angle to a typical setting for a Cellbag bioreactor at maximum volume (e.g., a rocking speed of 25 rpm and an angle of 7°).
4. Turn on the heater or place in an incubator at the normal operating temperature.
5. Allow the PBS and lipid solution to mix overnight.
6. Pump out or drain the PBS lipid solution the next day and add media and cells. Include the appropriate amounts of lipid supplement following the usual protocol.

Comments

Some adjustment of the lipid supplement concentration in the PBS and in the subsequent culture might be required. Additional lipid feeds of the culture can also help maintain the needed levels of lipid in the media.

Please contact GE Healthcare for the latest guidelines.

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Perfusion cell culture using WAVE Bioreactor Systems 2/10 and 20/50

Application

Perfusion cell culture has long been used as a method of achieving higher cell densities and increased culture length compared to batch culture methods. The greater cell density and longer culture life can result in better yields of secreted products, more efficient use of media, and the ability to generate large numbers of cells in small volumes. The greatest challenge of perfusion cell culture has always been the need to contain the cells within the culture without reducing cell viability.

The WAVE Bioreactor™ system provides a simple and reliable perfusion method using a disposable Cellbag™ bioreactor with an integral perfusion filter. The key to this perfusion system is the neutral buoyancy filter in the Cellbag bioreactor. This 'lily pad' filter floats just under the surface of the media where the wave action that mixes and oxygenates the culture helps wash the filter. The gentle washing prevents the filter from clogging and extends the length of the perfusion culture while maintaining a low-shear environment for the cells. Perfusion Cellbag bioreactors are available in 2, 10, 20, and 50 L sizes.

Operation

Before installing the perfusion Cellbag bioreactor on the WAVE Bioreactor platform, aseptically attach a harvest line to the Luer-Lok™ connector on the 'Y' fitting leading from the perfusion filter and attach a feed line to one of the other connections on the Cellbag bioreactor. The other connector on the 'Y' fitting is a Clave™ sample port for sampling the perfusate. In all other respects, start the culture as a normal batch run. Monitor cell density, viability, and metabolism while the cells are growing. Add media when required. For more detailed instructions, refer to the specific cell line protocol.

When the desired volume has been reached and when the cells are in the log growth phase, begin perfusion by starting the feed pump and enabling the harvest pump. Monitor the cell density, viability, and metabolism. Match the cell density and metabolism with the perfusion rate. Increase the perfusion rate as the cell density increases. Oxygen limitation can become an issue as the density of the culture increases. Monitor the oxygen levels in the culture and increase the rocking speed as required. It might be necessary to increase the oxygen levels in the headspace of the Cellbag bioreactor to maintain the appropriate oxygen amounts in the culture.

Use the optional integral Loadcell to control the harvest pump when using a System 20/50EHT. The Loadcell will automatically turn the harvest pump on and off to maintain the desired volume. The feed pump is controlled independently and must be set manually by the user. Pumps are not provided with the WAVE Bioreactor system, but can be obtained separately (PUMP20). When using a System 2/10EH, use the optional Perfusion Controller (PERFCONT2E), which contains feed and harvest pumps that are controlled by the user.

It is also possible to use a perfusion Cellbag bioreactor to concentrate cells before harvest or to remove media for batch feeding. Care must be taken not to pump the media out too fast. Excessive harvest speed will reduce filter life and might introduce air into the filter, which will reduce efficiency. The number of times that a culture can be concentrated is limited and depends on cell density and harvest rate. Do not concentrate the cells below 10% of the Cellbag bioreactor working volume.

Culture parameters

Rocking speed

The rocking speed is dependent on the culture volume, cell density, and Cellbag bioreactor size. Generally, increase the rocks per minute (rpm) as the oxygen demand increases. For additional information use the specific cell line protocol. Keep in mind that the high cell densities in perfusion culture might require higher rocking speeds than those used for batch culture. Monitor the oxygen levels and adjust as needed. Observe the cells to determine whether increased rpm is reducing cell viability.

Rocking angle

Increase the rocking angle and decrease speed if excessive foaming is observed. Experience shows that this will help to break up the foam. It is important to monitor the

oxygen levels of the culture and adjust the rocking angle as needed. High cell densities during perfusion might require a greater rocking angle than in batch culture. For additional information use the specific cell line protocol.

Aeration rate

The Cellbag bioreactor should be kept rigidly inflated. During inflation, a flow rate of up to 0.5 liters per minute (Lpm) can be used. Keep the filters clamped during the initial stages of the culture. Once vigorous growth is observed, set the flow rate to 0.1 Lpm for the Cellbag-2L, 0.2 Lpm for Cellbag-10L and -20L, and 0.3 Lpm for Cellbag-50L during the run. An O_2/Air Mix Plug-in Controller (O2MIX20) or a WAVEPOD™ Controller are available to provide precise oxygen levels in the headspace during a cultivation. Alternatively, a premixed cylinder of oxygen and nitrogen may be sourced from a laboratory gas supplier. Attach a source line to the air inlet of an airpump ensuring that the supply pressure to the airpump does not exceed 1 to 3 psig. At a flow rate of 0.2 Lpm a standard cylinder should last approximately 1 month.

Temperature

The typical operating temperature is 36°C to 37°C for mammalian cells and 27°C to 28°C for insect culture.

pH control

Monitor the pH of the culture carefully during perfusion. Changing the feed rate can change the pH. Increase or reduce the feed rate in small increments to prevent large changes in pH. Monitor the pH after feed rate changes and adjust the amount of CO_2 in the headspace if required. Reduce the amount of CO_2 as the pH drops and increase the amount of CO_2 as the pH increases. Most bicarbonate buffered media uses between 0% and 10% CO_2 in the headspace with 5% CO_2 being the typical maximum amount.

Feed rate

Perfusion should begin once the final culture volume is reached and the cells have exhibited strong growth. Depending on the growth rate of the cells, initial feed rates should be between 0.2 and 0.5 culture volumes per day. Monitor the nutrient levels, waste levels, and cell density. Continue to increase the feed rates by 0.2 to 0.5 culture volume increments every 24 to 48 h, as needed. The goal is to continue to add enough fresh media to maintain cell growth. Stop increasing the feed rate when the cell density stabilizes or the maximum feed rate desired is reached. Maximum feed rates of up to 3.0 culture volumes per day might be needed.

Scale up

A typical perfusion scheme for suspension-adapted CHO cells in a perfusion Cellbag-20L using the System 20/50EHT is given below. Keep in mind that this is a general guide only.

1. Following the protocol for batch culture, bring the perfusion Cellbag-20L to a volume of 10 L with a cell density of 0.5×10^6 viable cells per mL. The rocking speed should be 25 rpm and the angle at 7°. Maintain CO_2 levels at 5%.
2. After 1 to 2 days of cultivation (depending on doubling rate), the cell count should reach over 2×10^6 cells/mL. Monitor the glucose levels in the culture. As the glucose concentration falls below 2.0 g/L, begin the feed pump at 2 L/day (~ 1.4 mL/min). Enable the harvest pump.
3. Continue the culture for 24 to 48 h until the glucose concentration again falls below 2.0 g/L. Cell density should be about 4×10^6 viable cells/ml. Increase the feed rate to 7 L/day (4.9 mL/min). Increase the speed to 28 rpm. Monitor the pH because it will begin to drop.
4. After another day or two, increase the feed rate 15 L/day (8.3 mL/min) based on the glucose concentration. Cell density should be over 8×10^6 viable cells/mL. The pH will have dropped below pH 7.0 at this point. Reduce the CO_2 concentration to zero. Increase the angle to 9°.
5. Cell density will begin to plateau within 48 h. Monitor the glucose and increase or reduce the feed rate to maintain the desired glucose concentration. Feed rates generally top out at 2 to 3 culture volumes per day. Increase the rpm in response to oxygen demand. The pH will stabilize as the culture reaches a steady state. For CHO cells, the pH may be quite low at maximum cell density.

Comments

Do not rock too slowly. If growth appears poor, try raising the rocking speed by 3 to 5 rpm and observe cell growth after 24 h. Ideally, check the dissolved oxygen using a dissolved oxygen (DOOPT) system or WAVEPOD Controller.

The key to successful perfusion culture is to determine what benchmarks to use when deciding how much to adjust the feed rate. Cell density, pH, and nutrient or waste concentrations are all ways of inferring cellular metabolism. For CHO cells, glucose is a convenient indicator of metabolic activity. Optimizing media usage and product expression in a perfusion culture can be a long process. Multiple parameters can be adjusted together or independently to achieve different results.

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Cultivation of CHO and HEK293 cells in PEI transfection reagent using WAVE Bioreactor Systems 2/10 and 20/50

Application

Polyethylenimine (PEI) is a fast, effective, and inexpensive reagent for large-scale transient transfection. This method has been shown to express recombinant proteins in various media, with or without the presence of serum, using both HEK293 and CHO cultures (1).

PEI condenses plasmid DNA into positively charged particles that interact with the anionic cell surface. After entering the cell through endocytosis, the high charge density of the polymer causes lysosomal rupturing. This reduces degradation of the plasmid and releases it into the cytosol, allowing migration to the nucleus.

Preparation

Prior to transfecting, ensure the cell line is compatible with the plasmid vector and contains the desired components necessary for episomal replication etc. The plasmid should be sequence confirmed and checked for purity by absorbance (A_{260}/A_{280} ratio of > 1.8).

Inoculation

Inflate the Cellbag™ bioreactor with air and 10% CO_2 until it is rigid, add media and clamp the inlet and exhaust filters. Start rocking at 15 rocks per minute (rpm) and an angle of 7°. Allow the temperature and pH to equilibrate. Add cells to the Cellbag bioreactor. Cell inoculum should be sufficient to give a starting cell density of at least 0.5×10^6 cells/mL during initial runs. With CHO cells in particular, low initial cell densities can result in an excessive lag in cell growth. CHO cells can lag for several days after a passage into fresh media. Try to limit the size of the dilution factor and maintain an appropriate cell density when adding cells to the Cellbag bioreactor. Adjust the rpm and angle as described in "Culture parameters." Due to the efficient gas exchange that is possible with WAVE Bioreactor™ systems, the pH of bicarbonate buffered media can shift suddenly. Keeping the filters clamped during the early stages of the culture helps maintain a steady pH.

Grow enough cells to produce a culture that is between 0.5×10^6 and 1×10^6 cells/mL at the desired target volume. Viability should be 90% to 95%, or within 5% of the typical viability seen for that particular culture. Transfections have been successful using inoculum cultures of up to ~ 3.5×10^6 cells/mL before dilution. It is best to use cells in growth phase; maximum cell density will vary for different cultures (1). Choose a suitable media; a low calcium media will reduce aggregation (2) and 1% serum will improve transfection efficiency.

Materials

1. Linear 25 kD PEI (prepared as described in reference 2)
2. Plasmid DNA (typically ~ 1 mg/mL in Tris-EDTA buffer)
3. Fresh media (reduce or remove antibiotics, selection reagents, heparin, and other components of the media that interfere with transfection)

Culture parameters

Rocking speed

Use the maximum rocking speed the culture will tolerate. Increased agitation has been shown to be a useful way to reduce aggregation (3).

Rocking angle

Use the maximum rocking angle the culture will tolerate to reduce aggregation.

Transfection

Culture

Seed the cells at 1 to 2 × 10^6 cells/mL in half the target volume. When the culture is brought to full volume, the density will be 0.5 to 1 × 10^6 cells/mL.

DNA complex

Prepare the DNA complex using 2.5 ug of plasmid DNA per mL of culture. Calculate the amount of PEI necessary for a 1:3 DNA to PEI ratio (w/w), and combine in 150 mM NaCl, volume equal to 5% culture volume. Incubate for 10 min before adding to culture (1,4).

1. Prepare and add the complex 1 h after cell seeding.
2. Incubate the culture for 4 h, then bring the culture to full volume by adding an equal volume of media (1:2 dilution, final cell density 0.5 to 1 × 10^6).
3. Harvest the culture or media 4 to 7 days post transfection.

Comments

1. The health of the culture is critical for successful transfection and expression of protein. Check culture data often, using the following approximate values as guidelines:
   - Viability > 90% viable cells, growth phase
   - pH between 6.8 and 7.2
   - Lactate < 1.5 g/L
   - Glutamine > 1 mmol
   - Glucose > 1 g/L
2. Store the PEI reagent frozen or make fresh.
3. Aside from typical expression optimization (harvest time, media, vector etc.), other parameters can be optimized such as PEI preparation method, conditions for DNA:PEI complexing, DNA:PEI ratio. Resuspending the culture in fresh media prior to transfection can improve expression.
4. When expressing antibodies, the total weight of both plasmids (heavy and light chain constructs) should be reduced by 50% in order to maintain the same total amount required in the protocol.
5. For optimization experiments, 1% to 2% GFP containing plasmid can be added with the gene of interest to monitor transfection efficiency.
6. Other reagents, such as Fugen6™, can be used as a control. Protein expression will vary in different cultures, vectors, and with different reagents. Testing multiple methods will increase overall success.

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Cultivation of CHO cells in suspension using WAVE Bioreactor Systems 2/10 and 20/50

Application

Cultivation of suspension CHO cells for protein production is a popular application for the WAVE Bioreactor™ system. The cells are easy to grow and can be scaled up readily using a Cellbag™ disposable bioreactor. The volume in the Cellbag bioreactor can be increased by a factor of 10, reducing inoculum requirements and the need for transfers. For example, a Cellbag-20L can be started with as little as 1 L of culture and fresh media can be added to match growth to bring the final batch volume to 10 L. It is important to fully adapt CHO cells to suspension before attempting to grow them in the Cellbag bioreactor. Excessive cell clumping and attachment of cells to the Cellbag walls can result from incomplete adaptation to suspension culture.

Inoculation

Inflate the Cellbag bioreactor with air and 10% CO_2 until it is rigid, add media and clamp the inlet and exhaust filters. Start rocking at 15 rocks per minute (rpm) and an angle of 7°. Allow the temperature and pH to equilibrate. Add cells to the Cellbag bioreactor. Cell inoculum should be sufficient to give a starting cell density of at least 0.5×10^6 cells/mL during initial runs. Low initial cell densities can result in an excessive lag in cell growth. CHO cells can lag for several days after a passage into fresh media. Try to limit the size of the dilution factor and maintain an appropriate cell density when adding cells to a Cellbag bioreactor. Adjust the rpm and angle as described in "Culture parameters." Due to the efficient gas exchange that is possible with the WAVE Bioreactor system, the pH of bicarbonate buffered media can shift suddenly. Keeping the filters clamped during the early stages of the culture helps maintain a steady pH. Refer to "pH control" for some recommended techniques.

Operation

While the cells are growing, monitor cell density, viability, and metabolism adding media when required. If possible add media that has been warmed to the operating temperature. Keep the Cellbag bioreactor rigidly inflated at all times. When using a System 2/10EH or System 20/50EHT, make sure that there is good contact between the temperature probe and the culture. This will prevent overheating, which is particularly important if the culture is at a low volume. The oxygen levels of the culture are critical to successful cell growth and production. Monitor the oxygen levels with a dissolved oxygen (DOOPT) system, WAVEPOD™ Controller, or offline blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture.

Culture parameters

Rocking speed

The rocking speed is dependent on the culture volume, cell density, and Cellbag bioreactor size. Generally, increase the rpm as the oxygen demand increases. For Cellbag-2L, -10L, and -20L set at 15 to 20 rpm initially. For very low volumes (10% to 20% of the working volume), an initial speed of 15 rpm is sufficient. Increase the speed to 20 to 25 rpm as more media is added to the culture. When at 100% of the Cellbag bioreactor working volume or at high cell density, the speed might need to be as high as 25 rpm. For the Cellbag-50L, set the speed at 12 to 18 rpm initially. At 10% to 20% of the working volume, set the speed at 12 rpm. When at maximum volume, use a speed of 22 rpm. These are general guidelines only. Monitor the oxygen levels and adjust the rpm and rocking angle as needed. CHO cells are a hardy cell line and generally able to withstand a relatively high rocking speed.

Rocking angle

For the Cellbag-2L, -10L, and -20L an initial angle of 6° is sufficient. For Cellbag-50L, use an initial angle of 5°. To increase oxygen transfer when the Cellbag bioreactor is at 100% of its working volume, an angle of 7° or 8° might be needed when using Cellbag-2L, -10L, or -20L. For the Cellbag-50L, an angle of 6° to 7° may be used when at maximum volume. Increase the rocking angle and decrease speed if excessive foaming is observed. Experience shows that this will help to break up the foam. It is important to monitor the oxygen levels of the culture and adjust the rocking angle as needed.

Aeration rate

The Cellbag bioreactor should be kept rigidly inflated. During inflation, a flow rate of up to 0.5 liters per minute (Lpm) can be used. Keep the filters clamped during the initial stages of the culture. Once vigorous growth is observed, set the flow rate to 0.1 Lpm for the Cellbag-2L, 0.2 Lpm for Cellbag-10L and Cellbag-20L, and 0.3 Lpm for Cellbag-50L during the run.

Temperature

The typical operating temperature for CHO cells is 36°C to 37°C.

pH control

pH control is critical. Due to the high gas transfer capacity of the WAVE Bioreactor system, pH can drift rapidly. Use the following procedure:

1. Initially, inflate the Cellbag bioreactor with 10% CO_2/air. After inflation, add media into the bioreactor and close off the inlet and outlet air filters. Rock at 15 rpm for 1 to 2 h to allow the pH and temperature to completely equilibrate. Before inoculation, check the pH by taking a sample. Adjust if necessary.
2. Inoculate with cells. Leave the inlet and outlet filters closed.
3. Monitor pH, glucose concentration, and cell density. Once the pH and glucose levels start dropping, switch to 5% CO_2/air with continuous airflow through the headspace. This should occur within 24 to 60 h. Once vigorous cell growth occurs, the media pH will not drift upwards and CO_2 concentration in the sweep gas can be used to control pH.
4. Increase the rocking speed and angle to maintain oxygen concentration. Use offline sampling, a dissolved oxygen (DOOPT) system, or WAVEPOD Controller to estimate dissolved oxygen concentration.

Scale up

A typical scale up for CHO cells in a Cellbag-20L is given below. Keep in mind that this is a general guide only. Different cell lines behave differently.

1. Fill 1 L of media into a Cellbag-20L. Add inoculum to give a starting cell count of at least 0.5×10^6 cells/mL. Set the rocking speed to 15 rpm and the angle at 6°. Keep the system at operating temperature.
2. After 2 to 3 days of cultivation (depending on doubling rate), the cell count should reach over 2×10^6 cells/mL. At this stage, add 4 L of fresh media to bring the total volume to 5 L. Increase the speed to 20 rpm.
3. Continue the culture for a few more days until the cells reach about 2×10^6 cells/mL. Now add 5 L of fresh media. Increase the speed to 25 rpm and the angle to 7°. Monitor the oxygen levels in the culture carefully.
4. Continue the culture for a few more days, feeding the culture if desired, until cell viability begins to drop. Determine ahead of time the optimum viability at which to harvest.

For other size Cellbag bioreactors, adjust the volume proportionally. A Cellbag-10L would start at 500 mL and Cellbag-2L at 100 mL. When using a Cellbag-50L with a working volume of 25 L, use a slightly reduced rpm and angle when compared to the settings used for smaller Cellbags. For example, use a maximum speed of 23 rpm and angle of 6° with a Cellbag-50L rather then the settings given above.

Comments

Do not rock too slowly. If growth appears poor, try raising the rocking speed by 3 to 5 rpm and observe cell growth after 24 h. Ideally, check the dissolved oxygen using a dissolved oxygen (DOOPT) system or WAVEPOD Controller.

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Cultivation of CHO cells in suspension using WAVE Bioreactor System 200

Application

Cultivation of suspension CHO cells for protein production is a popular application for the WAVE Bioreactor™ system. The cells are easy to grow, and can be scaled up readily. The volume in the Cellbag™ disposable bioreactor can be increased by a factor of 10, reducing inoculum requirements and the need for transfers. For example, a Cellbag-200L can be started with as little as 10 L of culture and fresh media can be added to match growth to bring the final batch volume to 100 L. It is important to fully adapt CHO cells to suspension before attempting to grow them in the Cellbag bioreactor. Excessive cell clumping and attachment of cells to the Cellbag bioreactor can result from incomplete adaptation to suspension culture.

Inoculation

Inflate the Cellbag bioreactor with air and 10% CO_2 until it is rigid, add media and clamp the inlet and exhaust filters. Start rocking at 15 rocks per minute (rpm) and an angle of 7°. Allow the temperature and pH to equilibrate for several hours. Add cells to the Cellbag bioreactor. Cell inoculum should be sufficient to give a starting cell density of at least 5×10^5 cells/mL. Use a greater cell density if required by the cell line. Adjust the rpm and angle as described in "Culture parameters." Due to the efficient gas exchange that is possible with the WAVE Bioreactor system, the pH of bicarbonate buffered media can shift suddenly. Keeping the filters clamped during the early stages of the culture helps maintain a steady pH. Refer to "pH control" for some recommended techniques.

Operation

While the cells are growing, monitor cell density, viability, and metabolism adding media when required. If possible, add media that has been warmed to operating temperature. Room temperature media can also be used. The WAVE Bioreactor System 200 will rapidly warm the culture. Keep the Cellbag bioreactor rigidly inflated at all times. The oxygen levels of the culture are critical to successful cell growth and production. Monitor the oxygen levels with a dissolved oxygen (DOOPT) system or offline blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture.

Culture parameters

Rocking speed

The rocking speed is dependent on both the culture volume and cell density. Generally, increase the rpm as the oxygen demand increases. Set at 12 to 15 rpm initially. For very low volumes (10% to 20% of the working volume), an initial speed of 12 rpm is sufficient. Increase the speed to 15 to 20 rpm as more media is added to the culture. When at 100% of the Cellbag bioreactor working volume or at high cell density, the speed might need to be as high as 25 rpm. These are general guidelines only. Monitor the oxygen levels and adjust the rpm and rocking angle as needed. CHO cells are a hardy cell line and generally able to withstand a relatively high rocking speed.

Rocking angle

An initial angle of 6° is sufficient. To increase oxygen transfer when the Cellbag bioreactor is at 100% of the working volume, an angle of 7° or 8° might be needed. Increase the rocking angle and decrease speed if excessive foaming is observed. Experience shows that this will help to break up the foam. It is important to monitor the oxygen levels of the culture and adjust the rocking angle as needed.

Aeration rate

The Cellbag bioreactor should be kept rigidly inflated. During inflation, a flow rate of up to 5 liters per minute (Lpm) can be used. Keep the filters clamped during the initial stages of the culture. Once vigorous growth is observed, aeration can be set at 2 Lpm minimum. Flow rates up to 3 Lpm are commonly used.

Temperature

The typical operating temperature for CHO cells is 36°C to 37°C.

pH control

pH control is critical. Due to the high gas transfer capacity of the WAVE Bioreactor system, pH can drift rapidly. Use the following procedure:

1. Initially, inflate the Cellbag bioreactor with 10% CO_2/air. After inflation, add media and close off the inlet and outlet air filters. Rock at 15 rpm for 1 to 2 h to allow the pH and temperature to completely equilibrate. Before inoculation, check the pH by taking a sample. Adjust if necessary.
2. Inoculate with cells. Leave the inlet and outlet filters closed.
3. Monitor pH, glucose concentration, and cell density. Once the pH and glucose levels start dropping, switch to 5% CO_2/air with continuous airflow through the headspace. This should occur within 24 to 60 h. Once you get vigorous cell growth, the media pH will not drift upwards and CO_2 concentration in the sweep gas can be used to control pH.
4. Increase the rocking speed and angle to maintain oxygen concentration. Use offline sampling or a dissolved oxygen (DOOPT) system to estimate dissolved oxygen concentrations.

Scale up

A typical scale up for CHO cells in a Cellbag-200L is given below. Keep in mind that this is a general guide only. Different cell lines behave differently.

1. Fill 10 L of media into a Cellbag-200L. Add inoculum to give a starting cell count of at least 0.5×10^6 cells/mL. Set the rocking speed to 12 rpm and the angle at 6°. Keep the system at the operating temperature.
2. After 2 to 3 days of cultivation (depending on doubling rate), the cell count should reach over 2×10^6 cells/mL. At this stage, add 40 L of fresh media to bring the total volume to 50 L. Increase the speed to 18 rpm.
3. Continue the cell culture for a few more days until the cells reach about 2×10^6 cells/mL. Now add 50 L of fresh media. Increase the speed to 22 rpm and the angle to 7°. Monitor the oxygen levels in the culture carefully.
4. Continue the culture for a few more days, feeding the culture if desired, until cell viability begins to drop. Determine ahead of time the optimum viability at which to harvest.

For other size Cellbags adjust the volume proportionally. A Cellbag-100L would start at 5 L.

Comments

Do not rock too slowly. If growth appears poor, try raising the rocking speed by 3 to 5 rpm and observe cell growth after 24 h. Ideally, check the dissolved oxygen online using a dissolved oxygen (DOOPT) system.

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Cultivation of HEK 293 cells in suspension using WAVE Bioreactor Systems 2/10 and 20/50

Application

Cultivation of HEK 293 cells for virus and recombinant protein production is a popular application for the WAVE Bioreactor™ system. The cells are easily adapted to suspension culture in commercially available serum-free media and can be scaled up readily using a Cellbag™ bioreactor. The Cellbag bioreactor is completely closed and disposable making it ideal for viral production, both in terms of containment and cleanup. Furthermore, the volume in the Cellbag bioreactor can be increased by a factor of 10, reducing inoculum requirements and the need for transfers. For example, a Cellbag-20L can be started with as little as 1 L of culture and fresh media can be added to match growth to bring the final batch volume to 10 L.

Inoculation

Inflate the Cellbag bioreactor with air and 10% CO_2 until it is rigid, add media and clamp the inlet and exhaust filters. Start rocking at 15 rocks per minute (rpm) and an angle of 7°. Allow the temperature and pH to equilibrate. Add cells to the Cellbag bioreactor. Cell inoculum should be sufficient to give a starting cell density of at least 0.5×10^6 cells/mL during initial runs. Use a greater cell density if required. Adjust the rpm and angle as described in "Cuture parameters." Due to the efficient gas exchange that is possible with the WAVE Bioreactor system, the pH of bicarbonate buffered media can shift suddenly. Keeping the filters clamped during the early stages of the culture helps maintain a steady pH. Refer to "pH control" for some recommended techniques.

Operation

While the cells are growing, monitor cell density, viability, and metabolism adding media when required. If possible, add media that has been warmed to the operating temperature. Keep the Cellbag bioreactor rigidly inflated at all times. When using a System 2/10EH or 20/50EHT, make sure that there is good contact between the temperature probe and the culture. This will prevent overheating, which is particularly important if the culture is at a low volume. The oxygen levels of the culture are critical to successful cell growth and production. Monitor the oxygen levels with a dissolved oxygen (DOOPT) system, WAVEPOD™ Controller, or offline blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture.

Culture parameters

Rocking speed

The rocking speed is dependent on the culture volume, cell density, and Cellbag bioreactor size. Generally, increase the rpm as the oxygen demand increases. For Cellbag-2L, -10L, and -20L set at 12 to 20 rpm initially. For very low volumes (10% to 20% of the working volume), an initial speed of 12 rpm is sufficient. Increase the speed to 20 to 25 rpm as more media is added to the culture. When at 100% of the Cellbag bioreactor working volume or at high cell density, the speed might need to be as high as 25 rpm. For the Cellbag-50L, set the speed at 10 to 18 rpm initially. At 10% to 20% of the working volume, set the speed at 10 rpm. When at maximum volume, use a speed of 22 rpm. These are general guidelines only. Monitor the oxygen levels and adjust the rpm and rocking angle as needed.

Rocking angle

For the Cellbag-2L, -10L, and -20L an initial angle of 6° is sufficient. For Cellbag-50L, use an initial angle of 5°. To increase oxygen transfer when the Cellbag bioreactor is at 100% of the working volume, an angle of 7° or 8° might be needed when using Cellbag-2L, -10L, and -20L. For the Cellbag-50L, an angle of 6° to 7° may be used when at maximum volume. Increase the rocking angle and decrease speed if excessive foaming is observed. Experience shows that this will help to break up the foam. It is important to monitor the oxygen levels of the culture and adjust the rocking angle as needed.

Aeration rate

The Cellbag bioreactor should be kept rigidly inflated. During inflation, a flow rate of up to 0.5 liters per minute (Lpm) can be used. During the initial stages of the culture, keep the filters clamped. Once vigorous growth is observed, set the flow rate to 0.1 Lpm for the Cellbag-2L, 0.2 Lpm for Cellbag-10L and Cellbag-20L, and 0.3 Lpm for Cellbag-50L during the run.

Temperature

The typical operating temperature for HEK293 cells is 36°C to 37°C.

pH control

pH control is critical. Due to the high gas transfer capacity of the WAVE Bioreactor system, pH can drift rapidly. Use the following procedure:

1. Initially, inflate the Cellbag bioreactor with 10% CO_2/air. After inflation, add media into the bioreactor and close off the inlet and outlet air filters. Rock at 15 rpm for 1 to 2 h to allow the pH and temperature to completely equilibrate. Before inoculation, check the pH by taking a sample. Adjust if necessary.
2. Inoculate with cells. Leave the inlet and outlet filters closed.
3. Monitor pH, glucose concentration, and cell density. Once the pH and glucose levels start dropping, switch to 5% CO_2/air with continuous airflow through the headspace. This should occur within 24 to 60 h. Once vigorous cell growth occurs, the media pH will not drift upwards and CO_2 concentration in the sweep gas can be used to control pH.
4. Increase the rocking speed and angle to maintain oxygen concentration. Use offline sampling, a DOOPT system, or WAVEPOD Controller to estimate dissolved oxygen concentration.

Scale up

A typical scale up for HEK293 cells in a Cellbag-20L is given below. Keep in mind that this is a general guide only.

1. Fill 1 L of media into a Cellbag-20L. Add inoculum to give a starting cell count of at least 0.5×10^6 cells/mL. Set the rocking speed to 12 rpm and the angle at 6°. Keep the system at the operating temperature.
2. After 2 to 3 days of cultivation (depending on doubling rate), the cell count should reach about 2×10^6 cells/mL. At this stage, add 4 L of fresh media to bring the total volume to 5 L. Increase the speed to 20 rpm.
3. Continue the culture for a few more days until the cells reach 1.5 to 2×10^6 cells/mL. Now add 5 L of fresh media. Increase the speed to 25 rpm and the angle to 7°. Monitor the oxygen levels in the culture carefully.
4. When the desired cell density has been reached add virus at the appropriate MOI.
5. Continue the culture for another day or two until cell viability begins to drop. Determine ahead of time the optimum viability at which to harvest.

For other size Cellbag bioreactors, adjust the volume proportionally. A Cellbag-10L would start at 500 mL and Cellbag-2L at 100 mL. When using a Cellbag-50L with a working volume of 25 L, generally use a slightly reduced rpm and angle when compared to the settings used for smaller Cellbag bioreactors. For example, use a maximum speed of 23 rpm and angle of 6° with a Cellbag-50L rather then the settings given above.

Comments

Do not rock too slowly. If growth appears poor, try raising the rocking speed by 3 to 5 rpm and observe cell growth after 24 h. Ideally, check the dissolved oxygen using a dissolved oxygen (DOOPT) system or WAVEPOD Controller.

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Cultivation of HEK 293 cells in suspension using WAVE Bioreactor System 200

Application

Cultivation of HEK 293 cells for recombinant protein and viral production is a popular application for the WAVE Bioreactor™ system. The cells are easily adapted to suspension in commercially available media and can be scaled up readily using a Cellbag™ disposable bioreactor. Because the Cellbag bioreactor is a closed system and completely disposable, it is ideally suited to applications where containment is important. Furthermore, the volume in a Cellbag bioreactor can be increased by a factor of 10, reducing inoculum requirements and the need for transfers. For example, a Cellbag-200L can be started with as little as 10 L of culture and fresh media can be added to match growth to bring the final batch volume to 100 L

Inoculation

Inflate the Cellbag bioreactor with air and 10% CO_2 until it is rigid, add media, and clamp the inlet and exhaust filters. Start rocking at 15 rocks per minute (rpm) and an angle of 7°. Allow the temperature and pH to equilibrate for several hours. Add cells to the Cellbag bioreactor. Cell inoculum should be sufficient to give a starting cell density of at least 0.5×10^6 cells/mL. Use a greater cell density if required by the cell line. Adjust the rpm and angle as described in "Culture parameters." Due to the efficient gas exchange that is possible with the WAVE Bioreactor system, the pH of bicarbonate buffered media can shift suddenly. Keeping the filters clamped during the early stages of the culture helps maintain a steady pH. Refer to "pH control" for some recommended techniques.

Operation

While the cells are growing, monitor density, viability, and metabolism adding media when required. If possible, add media that has been warmed to the operating temperature although room temperature media can also be used because the WAVE Bioreactor System 200 will rapidly warm the media to the appropriate temperature. Keep the Cellbag bioreactor rigidly inflated at all times. The oxygen levels of the culture are critical to successful cell growth and production. Monitor the oxygen levels with a dissolved oxygen (DOOPT) system or offline blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture.

Culture parameters

Rocking speed

The rocking speed is dependent on both the culture volume and cell density. Generally, increase the rpm as the oxygen demand increases. Set at 10 to 15 rpm initially. For very low volumes (10% to 20% of the working volume), an initial speed of 12 rpm is sufficient. Increase the speed to 15 to 20 rpm as more media is added to the culture. When at 100% of the Cellbag bioreactor working volume or at high cell density, the speed might need to be as high as 25 rpm. These are general guidelines only. Monitor the oxygen levels and adjust the rpm and rocking angle as needed.

Rocking angle

An initial angle of 6° is sufficient. To increase oxygen transfer when the Cellbag bioreactor is at 100% of the working volume, an angle of 8° or 9° might be needed. Increase the rocking angle and decrease speed if excessive foaming is observed. Experience shows that this will help to break up the foam. It is important to monitor the oxygen levels of the culture and adjust the rocking angle as needed.

Aeration rate

The Cellbag bioreactor should be kept rigidly inflated. During inflation, a flow rate of up to 5 liters per minute (Lpm) can be used. Keep the filters clamped during the initial stages of the culture. Once vigorous growth is observed, aeration can be set at a minimum of 2 Lpm. Flow rates of up to 3 Lpm are commonly used.

Temperature

The typical operating temperature for HEK 293 cells is 37ºC.

pH control

pH control is critical. Due to the high gas transfer capacity of the WAVE Bioreactor system, pH can drift rapidly. Use the following procedure:

1. Initially, inflate the Cellbag bioreactor with 10% CO_2/air. After inflation, add media into the bioreactor and close off the inlet and outlet air filters. Rock at 15 rpm for 1 to 2 h to allow the pH and temperature to completely equilibrate. Before inoculation, check the pH by taking a sample. Adjust if necessary.
2. Inoculate with cells. Leave the inlet and outlet filters closed.
3. Monitor pH, glucose concentration, and cell density. Once the pH and glucose levels start dropping, switch to 5% CO_2/air with continuous airflow through the headspace at a minimum of 2 Lpm. This should occur within 24 to 60 h. Once you get vigorous cell growth, the media pH will not drift upwards and CO_2 concentration in the sweep gas can be used to control pH.
4. Increase the rocking speed and angle to maintain oxygen concentration. Use offline sampling or a dissolved oxygen (DOOPT) system to estimate dissolved oxygen concentration.

Scale up

A typical scale up for HEK 293 cells in a Cellbag-200L is given below. Keep in mind that this is a general guide only.

1. Fill 10 L of media into a Cellbag-200L. Add inoculum to give a starting cell count of at least 0.5×10^6 cells/mL. Set the rocking speed to 12 rpm and the angle at 6º. Keep the system at the operating temperature.
2. After 2 to 3 days of cultivation (depending on doubling rate), the cell count should reach over 2×10^6 cells/mL. At this stage, add 40 L of fresh media to bring the total volume to 50 L. Increase the speed to 18 rpm.
3. Continue the cell culture for a few more days until the cells reach about 2×10^6 cells/mL. Now add 50 L of fresh media. Increase the speed to 25 rpm and the angle to 7º. Monitor the oxygen levels in the culture carefully.
4. Continue the culture for a few more days until the desired cell density is reached. Add virus at the appropriate MOI if desired. Continue the culture until cell viability begins to drop. Determine ahead of time the optimum viability at which to harvest.

For other size Cellbag bioreactors, adjust the volume proportionally. A Cellbag-100L would start at 5 L.

Comments

Do not rock too slowly. If growth appears poor, try raising the speed by 3 to 5 rpm and observe cell growth after 24 h. Ideally, check the dissolved oxygen online using a dissolved oxygen (DOOPT) system.

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Cultivation of hybridoma cells in suspension using WAVE Bioreactor Systems 2/10 and 20/50

Application

Cultivation of various hybridoma cells for the production of antibodies is a popular application for the WAVE Bioreactor™ system. The cells are easy to grow and can be scaled up readily using a Cellbag™ disposable bioreactor. The volume in the Cellbag bioreactor can be increased by a factor of 10, reducing inoculum requirements and the need for transfers. For example, a Cellbag-20L can be started with as little as 1 L of culture and fresh media can be added to match growth to bring the final batch volume to 10 L. Because of the variety of hybridoma parent cell lines and the differences that arise during the fusion process, the growth characteristics of hybridomas vary greatly. It is important to characterize the growth and production patterns of each cell line before scale up.

Inoculation

Inflate the Cellbag bioreactor with air and 10% CO_2 until it is rigid, add media and clamp the inlet and exhaust filters. Start rocking at 15 rocks per minute (rpm) and an angle of 7°. Allow the temperature and pH to equilibrate. Add cells to the Cellbag bioreactor. Cell inoculum should be sufficient to give a starting cell density of at least 0.5×10^6 cells/mL during initial runs. Use a greater cell density if required by the cell line. Adjust the rpm and angle as described in "Culture parameters." Due to the efficient gas exchange that is possible with the WAVE Bioreactor system, the pH of bicarbonate buffered media can shift suddenly. During the early stages of the culture keeping the filters clamped helps maintain a steady pH. Refer to "pH control" for some recommended techniques.

Operation

While the cells are growing, monitor cell density, viability, and metabolism adding media when required. If possible, add media that has been warmed to the operating temperature. Keep the Cellbag bioreactor rigidly inflated at all times. When using a System 2/10EH or 20/50EHT, make sure that there is good contact between the temperature probe and the culture. This will prevent overheating, which is particularly important if the culture is at a low volume. The oxygen levels of the culture are critical to successful cell growth and production. Monitor the oxygen levels with a dissolved oxygen (DOOPT) system, WAVEPOD™ Controller, or offline blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture.

Culture parameters

Rocking speed

The rocking speed is dependent on the culture volume, cell density, and Cellbag bioreactor size. Generally, increase the rpm as the oxygen demand increases. For Cellbag-2L, -10L, and -20L set at 12 to 20 rpm initially. For very low volumes (10% to 20% of the working volume), an initial speed of 12 rpm is sufficient. Increase the speed to 20 to 25 rpm as more media is added to the culture. When at 100% of the Cellbag bioreactor working volume or at high cell density, the speed might need to be as high as 25 rpm. For the Cellbag-50L set the speed at 10 to 18 rpm initially. At 10% to 20% of the working volume, set the speed at 10 rpm. When at maximum volume, use a speed of 22 rpm. These are general guidelines only. Monitor the oxygen levels and adjust the rpm and rocking angle as needed.

Rocking angle

For the Cellbag-2L, -10L, and -20L an initial angle of 6° is sufficient. For Cellbag-50L, use an initial angle of 5°. To increase oxygen transfer when the Cellbag bioreactor is at 100% of its working volume, an angle of 7° or 8° might be needed when using Cellbag-2L, -10L, and -20L. For the Cellbag-50L, an angle of 6° to 7° may be used when at maximum volume. Increase the rocking angle and decrease speed if excessive foaming is observed. Experience shows that this will help to break up the foam. It is important to monitor the oxygen levels of the culture and adjust the rocking angle as needed.

Aeration rate

The Cellbag bioreactor should be kept rigidly inflated. During inflation, a flow rate of up to 0.5 liters per minute (lpm) can be used. During the initial stages of the culture keep the filters clamped. Once vigorous growth is observed set the flow rate to 0.1 Lpm for the Cellbag-2L, 0.2 Lpm for Cellbag-10L and Cellbag-20L, and 0.3 Lpm for Cellbag-50L during the run.

Temperature

The typical operating temperature for hybridoma cells is 36°C to 37°C.

pH control

pH control is critical. Due to the high gas transfer capacity of the WAVE Bioreactor system, pH can drift rapidly. Use the following procedure:

1. Initially, inflate the Cellbag bioreactor with 10% CO_2/air. After inflation, add media into the bioreactor and close off the inlet and outlet air filters. Rock at 15 rpm for 1 to 2 h to allow the pH and temperature to completely equilibrate. Before inoculation, check the pH by taking a sample. Adjust if necessary.
2. Inoculate with cells. Leave the inlet and outlet filters closed.
3. Monitor pH, glucose concentration, and cell density. Once the pH and glucose levels start dropping, switch to 5% CO_2/air with continuous airflow through the headspace. This should occur within 24 to 60 h. Once vigorous cell growth occurs, the media pH will not drift upwards and CO_2 concentration in the sweep gas can be used to control pH.
4. Increase the rocking speed and angle to maintain oxygen concentration. Use offline sampling, a DOOPT system, or WAVEPOD Controller to estimate dissolved oxygen concentration.

Scale up

A typical scale up for a hybridoma cell line in a Cellbag-20L is given below. Keep in mind that this is a general guide only. Different cell lines behave differently.

1. Fill 1 L of media into a Cellbag-20L. Add inoculum to give a starting cell count of at least 0.5×10^6 cells/mL. Set the rocking speed to 12 rpm and the angle at 6°. Keep the system at operating temperature.
2. After 2 to 3 days of cultivation (depending on doubling rate), the cell count should reach over 2×10^6 cells/mL. At this stage, add 4 L of fresh media to bring the total volume to 5 L. Increase the speed to 20 rpm.
3. Continue the culture for a few more days until the cells reach about 2×10^6 cells/mL. Now add 5 L of fresh media. Increase the speed to 25 rpm and the angle to 7°. Monitor the oxygen levels in the culture carefully.
4. Continue the culture for a few more days, feeding the culture if desired, until cell viability begins to drop. Determine ahead of time the optimum viability at which to harvest.

For other size Cellbag bioreactors, adjust the volume proportionally. A Cellbag-10L would start at 500 mL and Cellbag-2L at 100 mL. When using a Cellbag-50L with a working volume of 25 L, generally use a slightly reduced rpm and angle when compared to the settings used for smaller Cellbag bioreactorss. For example use a maximum speed of 23 rpm and angle of 6° with a Cellbag-50L rather then the settings given above.

Comments

Do not rock too slowly. If growth appears poor, try raising the rocking speed by 3 to 5 rpm and observe cell growth after 24 h. Ideally, check the dissolved oxygen using a dissolved oxygen (DOOPT) system or WAVEPOD Controller,.

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Cultivation of hybridoma cells in suspension using WAVE Bioreactor System 200

Application

Cultivation of various hybridoma cells for the production of antibodies is a popular application for the WAVE Bioreactor™ system. The cells are easy to grow, and can be scaled up readily. Furthermore, the volume in the Cellbag™ disposable bioreactor can be increased by a factor of 10, reducing inoculum requirements and the need for transfers. For example, a Cellbag-200L can be started with as little as 10 L of culture and fresh media can be added to match growth to bring the final batch volume to 100 L. Because of the variety of hybridoma parent cell lines and the differences that arise during the fusion process, the growth characteristics of hybridomas vary greatly. It is important to characterize the growth and production patterns of each cell line before scale up.

Inoculation

Inflate the Cellbag bioreactor with air and 10% CO_2 until it is rigid, add media and clamp the inlet and exhaust filters. Start rocking at 15 rocks per minute (rpm) and an angle of 7°. Allow the temperature and pH to equilibrate for several hours. Add cells to the Cellbag. Cell inoculum should be sufficient to give a starting cell density of at least 0.5×10^6 cells/mL. Use a greater cell density if required by the cell line. Adjust the rpm and angle as described in "Culture parameters." Due to the efficient gas exchange that is possible with the WAVE Bioreactor system, the pH of bicarbonate buffered media can shift suddenly. Keeping the filters clamped during the early stages of the culture helps maintain a steady pH. Refer to "pH control" for some recommended techniques.

Operation

As the cells grow, monitor density, viability, and metabolism adding media when required. If possible, add media that has been warmed to the operating temperature although room temperature media can also be used because the WAVE Bioreactor System 200 will rapidly warm the culture to the appropriate temperature. Keep the Cellbag bioreactor rigidly inflated at all times. The oxygen levels of the culture are critical to successful cell growth and production. Monitor the oxygen levels with a dissolved oxygen (DOOPT) system, or offline blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture.

Culture parameters

Rocking speed

The rocking speed is dependent on both the culture volume and cell density. Generally, increase the rpm as the oxygen demand increases. Set the speed at 10 to 15 rpm initially. For very low volumes (10% to 20% of the working volume), an initial speed of 12 rpm is sufficient. Increase the speed to 15 to 20 rpm as more media is added to the culture. When at 100% of the Cellbag bioreactor working volume or at high cell density, the speed might need to be as high as 25 rpm. These are general guidelines only. Monitor the oxygen levels and adjust the rpm and rocking angle as needed.

Rocking angle

An initial angle of 6° is sufficient. To increase oxygen transfer when the Cellbag bioreactor is at 100% of the working volume, an angle of 8° or 9° might be needed. Increase the rocking angle and decrease speed if excessive foaming is observed. Experience shows that this will help to break up the foam. It is important to monitor the oxygen levels of the culture and adjust the rocking angle as needed.

Aeration rate

The Cellbag bioreactor should be kept rigidly inflated. During inflation, a flow rate of up to 5 liters per minute (Lpm) can be used. During the initial stages of the culture, keep the filters clamped. Once vigorous growth is observed, aeration can be set at a minimum of 2 Lpm. Flow rates up to 3 Lpm are commonly used.

Temperature

The typical operating temperature for hybridoma cells is 36°C to 37°C.

pH control

pH control is critical. Due to the high gas transfer capacity of the WAVE Bioreactor system, pH can drift rapidly. Use the following procedure:

1. Initially, inflate the Cellbag bioreactor with 10% CO_2/air. After inflation add media into the bioreactor and close off the inlet and outlet air filters. Rock at 15 rpm for 1 to 2 h to allow the pH and temperature to completely equilibrate. Before inoculation, check the pH by taking a sample. Adjust if necessary.
2. Inoculate with cells. Leave the inlet and outlet filters closed.
3. Monitor pH, glucose concentration, and cell density. Once the pH and glucose levels start dropping, switch to 5% CO_2/air with continuous airflow through the headspace at a minimum of 2.0 Lpm. This should occur within 24 to 60 h. Once you get vigorous cell growth, the media pH will not drift upwards and CO_2 concentration in the sweep gas can be used to control pH.
4. Increase the rocking speed and angle to maintain oxygen concentration. Use offline sampling or a dissolved oxygen (DOOPT) system to estimate dissolved oxygen concentrations.

Scale up

A typical scale up for a hybridoma cell line in a Cellbag-200L is given below. Keep in mind that this is a general guide only. Different cell lines behave differently.

1. Fill 10 L of media into a Cellbag-200L. Add inoculum to give a starting cell density of at least 0.5×10^6 cells/mL. Set the rocking speed to 12 rpm and the angle at 6°. Keep the system at the operating temperature.
2. After 2 to 3 days of cultivation (depending on doubling rate), the cell count should reach over 2×10^6 cells/mL. At this stage, add 40 L of fresh media to bring the total volume to 50 L. Increase the speed to 18 rpm.
3. Continue the cell culture for a few more days until the cells reach about 2×10^6 cells/mL. Now add 50 L of fresh media. Increase the speed to 25 rpm and the angle to 7°. Monitor the oxygen levels in the culture carefully.
4. Continue the culture for a few more days, feeding the culture if desired, until cell viability begins to drop. Determine ahead of time the optimum viability at which to harvest.

For other size Cellbag bioreactors, adjust the volume proportionally. A Cellbag-100L would start at 5 L.

Comments

Do not rock too slowly. If growth appears poor, try raising the rocking speed by 3 to 5 rpm and observe cell growth after 24 h. Ideally, check the dissolved oxygen online using a dissolved oxygen (DOOPT) system.

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Cultivation of NS0 cells in suspension using WAVE Bioreactor Systems 2/10 and 20/50

Application

Cultivation of NS0 cells for the production of recombinant proteins is a popular application for the WAVE Bioreactor™ system. The cells are easy to grow, and can be scaled up readily in a Cellbag™ disposable bioreactor. The volume in the Cellbag bioreactor can be increased by a factor of 10, reducing inoculum requirements and the need for transfers. For example, a Cellbag-20L can be started with as little as 1 L of culture and fresh media can be added to match growth to bring the final batch volume to 10 L. Most NS0 cells are cholesterol dependent. The media needs to contain a lipid source or a lipid supplement must be added. During scale up, it is important to characterize the lipid requirements of the cell line and optimize the lipid levels in the Cellbag bioreactor.

Inoculation

Inflate the Cellbag bioreactor with air and 10% CO_2 until it is rigid, add media and clamp the inlet and exhaust filters. Start rocking at 15 rocks per minute (rpm) and an angle of 6°. Allow the temperature and pH to equilibrate. Add cells to the Cellbag bioreactor. Cell inoculum should be sufficient to give a starting cell density of at least 0.5×10^6 cells/mL. Use a greater cell density if required by the cell line. Adjust the rpm and angle as described in "Culture parameters." Due to the efficient gas exchange that is characteristic of the WAVE Bioreactor system, the pH of bicarbonate buffered media can shift suddenly. Keeping the filters clamped during the early stages of the culture helps maintain a steady pH. Refer to "pH control" for some recommended techniques.

Operation

While the cells are growing monitor cell density, viability, and metabolism adding media when required. If possible, add media that has been warmed to operating temperature. Keep the Cellbag bioreactor rigidly inflated at all times. When using a System 2/10 or 20/50EHT, make sure that there is good contact between the temperature probe and the culture. This will prevent overheating, which is particularly important if the culture is at a low volume. The oxygen levels of the culture are critical to successful cell growth and production. Monitor the oxygen levels with a dissolved oxygen (DOOPT) system, WAVEPOD™ Controller, or offline blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture. Lipid availability can be a limiting factor in NS0 cultures. Providing a lipid feed 24 to 48 h after final volume is reached can be helpful in extending the culture life and increasing cell density.

Culture parameters

Rocking speed

The rocking speed is dependent on the culture volume, cell density, and Cellbag bioreactor size. Generally, increase the rpm as the oxygen demand increases. For Cellbag-2L, -10L, and -20L set at 12 to 20 rpm initially. For very low volumes (10% to 20% of the working volume), an initial speed of 12 rpm is sufficient. Increase the speed to 20 to 25 rpm as more media is added to the culture. When at 100% of the Cellbag working volume or at high cell density, the speed might need to be as high as 25 rpm. For Cellbag-50L, set the speed at 10 to 18 rpm initially. At 10% to 20 % of the working volume, set the speed at 10 rpm. When at maximum volume, use a speed of 22 rpm. These are general guidelines only. Monitor the oxygen levels and adjust the rpm and rocking angle as needed.

Rocking angle

For the Cellbag-2L, -10L, and -20L an initial angle of 6° is sufficient. For Cellbag-50L, use an initial angle of 5°. To increase oxygen transfer when the Cellbag bioreactor is at 100% of its working volume, an angle of 7° or 8° might be needed when using Cellbag-2L, -10L, or 20L. For the Cellbag50L, an angle of 6° to 7° may be used when at maximum volume. Increase the rocking angle and decrease speed if excessive foaming is observed. Experience shows that this will help to break up the foam. It is important to monitor the oxygen levels of the culture and adjust the rocking angle as needed.

Aeration rate

The Cellbag bioreactor should be kept rigidly inflated. During inflation, a flow rate of up to 0.5 liters per minute (Lpm) can be used. Keep the filters clamped during the initial stages of the culture. Once vigorous growth is observed, set the flow rate to 0.1 Lpm for the Cellbag-2L, 0.2 Lpm for Cellbag-10L and Cellbag-20L, and 0.3 Lpm for Cellbag-50L during the run.

Temperature

Typical operating temperature for NS0 cells is 36°C to 37°C.

pH control

pH control is critical. Due to the high gas transfer capacity of the WAVE Bioreactor system, pH can drift rapidly. Use the following procedure:

1. Initially, inflate the Cellbag bioreactor with 10% CO_2/air. After inflation, add media into the bioreactor and close off the inlet and outlet air filters. Rock at 15 rpm for 1 to 2 h to allow the pH and temperature to completely equilibrate. Before inoculation, check the pH by taking a sample. Adjust if necessary.
2. Inoculate with cells. Leave the inlet and outlet filters closed.
3. Monitor pH, glucose concentration, and cell density. Once the pH and glucose levels start dropping, switch to 5% CO_2/air with continuous airflow through the headspace. This should occur within 24 to 60 h. Once vigorous cell growth occurs, the media pH will not drift upwards and CO_2 concentration in the sweep gas can be used to control pH.
4. Increase the rocking speed and angle to maintain oxygen concentration. Use offline sampling, a DOOPT system, or WAVEPOD Controller to estimate dissolved oxygen concentrations.

Scale up

A typical scale up for NS0 cells in a Cellbag-20L is given below. Keep in mind that this is a general guide only. Different cell lines behave differently.

1. Fill 1 L of media into a Cellbag-20L. Add inoculum to give a starting cell count of at least 0.5×10^6 cells/mL. Set the rocking speed to 12 rpm and the angle at 6°. Keep the system at operating temperature.
2. After 2 to 3 days of cultivation (depending on doubling rate), the cell count should reach over 2×10^6 cells/mL. At this stage add 4 L of fresh media to bring the total volume to 5 L. Increase the speed to 20 rpm.
3. Continue the cell culture for a few more days until the cells reach about 2×10^6 cells/mL. Now add 5 L of fresh media. Increase the speed to 25 rpm and the angle to 7°. Monitor the oxygen levels in the culture carefully.
4. Continue the culture for a few more days, feeding the culture if desired, until cell viability begins to drop. Determine ahead of time the optimum viability at which to harvest.

For other size Cellbag bioreactors, adjust the volume proportionally. A Cellbag-10L would start at 500 mL and Cellbag-2L at 100 mL. When using a Cellbag-50L with a working volume of 25 L, generally use a slightly reduced rpm and angle when compared to the settings used for smaller Cellbag bioreactors. For example, use a maximum speed of 23 rpm and angle of 6° with a Cellbag-50L rather than the settings given above.

Comments

Do not rock too slowly. If growth appears poor, try raising the rocking speed by 3 to 5 rpm and observe cell growth after 24 h. Ideally, check the dissolved oxygen using a dissolved oxygen (DOOPT) system or WAVEPOD Controller.

Refer to *Cellbag pretreatment for the use of chemically defined lipid supplements* (28-9308-85) for the procedure on lipid pretreatment.

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Cultivation of NS0 cells in suspension using WAVE Bioreactor System 200

Application

Cultivation of NS0 cells for the production of recombinant proteins is a popular application for the WAVE Bioreactor™ system. The cells are easy to grow and can be scaled up readily using a Cellbag™ disposable bioreactor. Furthermore, the volume in the Cellbag bioreactor can be increased by a factor of 10, reducing inoculum requirements and the need for transfers. For example, a Cellbag-200L can be started with as little as 10 L of culture and fresh media can be added to match growth to bring the final batch volume to 100 L. Many NS0 cell lines are cholesterol dependent. It might be necessary for the media used to contain a lipid source or a lipid supplement must be added. During the scale up process, it is important to characterize the lipid requirements of the cell line and, if necessary, optimize the lipid levels in the media in the Cellbag bioreactor.

Inoculation

Inflate the Cellbag bioreactor with air and 10% CO_2 until it is rigid, add media, and clamp the inlet and exhaust filters. Start rocking at 15 rocks per minute (rpm) and an angle of 7°. Allow the temperature and pH to equilibrate for several hours. Add cells to the Cellbag bioreactor. Cell inoculum should be sufficient to give a starting cell density of at least 0.5×10^6 cells/mL. Use a greater cell density if required by the cell line. Adjust the rpm and angle as described in "Culture parameters." Due to the efficient gas exchange that is possible with the WAVE Bioreactor system, the pH of bicarbonate buffered media can shift suddenly. Keeping the filters clamped during the early stages of the culture helps maintain a steady pH. Refer to "pH control" for some recommended techniques.

Operation

While the cells are growing, monitor cell density, viability, and metabolism adding media when required. If possible, add media that has been warmed to the operating temperature although room temperature media can also be used because the WAVE Bioreactor System 200 will rapidly warm the culture to the appropriate temperature. Keep the Cellbag bioreactor rigidly inflated at all times. The oxygen levels of the culture are critical to successful cell growth and production. Monitor the oxygen levels with a dissolved oxygen (DOOPT) system or offline blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture.

Culture parameters

Rocking speed

The rocking speed is dependent on both the culture volume and cell density. Generally, increase the rpm as the oxygen demand increases. Set at 10 to 15 rpm initially. For very low volumes (10% to 20% of the working volume), an initial speed of 12 rpm is sufficient. Increase the speed to 15 to 20 rpm as more media is added to the culture. When at 100% of the Cellbag bioreactor working volume or at high cell density, the speed might need to be as high as 25 rpm. These are general guidelines only. Monitor the oxygen levels and adjust the rpm and rocking angle as needed.

Rocking angle

An initial angle of 6° is sufficient. To increase oxygen transfer when the Cellbag bioreactor is at 100% of the working volume, an angle of 8° or 9° might be needed. Increase the rocking angle and decrease speed if excessive foaming is observed. Experience shows that this will help to break up the foam. It is important to monitor the oxygen levels of the culture and adjust the rocking angle as needed.

Aeration rate

The Cellbag bioreactor should be kept rigidly inflated. During inflation, a flow rate of up to 5 liters per minute (Lpm) can be used. Keep the filters clamped during the initial stages of the culture. Once vigorous growth is observed, aeration can be set at 2 Lpm minimum. Flow rates up to 3 Lpm are commonly used.

Temperature

The typical operating temperature for NS0 cells is 36°C to 37°C.

pH control

pH control is critical. Due to the high gas transfer capacity of the WAVE Bioreactor system, pH can drift rapidly. Use the following procedure:

1. Initially, inflate the Cellbag bioreactor with 10% CO_2/air. After inflation, add media into the bioreactor and close off the inlet and outlet air filters. Rock at 15 rpm for 1 to 2 h to allow the pH and temperature to completely equilibrate. Before inoculation, check the pH by taking a sample. Adjust if necessary.
2. Inoculate with cells. Leave the inlet and outlet filters closed.
3. Monitor pH, glucose concentration, and cell density. Once the pH and glucose levels start dropping, switch to 5% CO_2/air with continuous airflow through the headspace at a minimum of 2 Lpm. This should occur within 24 to 60 h. Once vigorous cell growth occurs, the media pH will not drift upwards and CO_2 concentration in the sweep gas can be used to control pH.
4. Increase the rocking speed and angle to maintain oxygen concentration. Use offline sampling or a dissolved oxygen (DOOPT) system to estimate dissolved oxygen concentration.

Scale up

A typical scale up for NS0 cells in a Cellbag-200L is given below. Keep in mind that this is a general guide only. Different cell lines behave differently.

1. Fill 10 L of media into a Cellbag-200L. Add inoculum to give a starting cell count of at least 0.5×10^6 cells/mL. Set the rocking speed to 12 rpm and the angle at 6°. Keep the system at operating temperature.
2. After 2 to 3 days of cultivation (depending on doubling rate), the cell count should reach over 2×10^6 cells/mL. At this stage, add 40 L of fresh media to bring the total volume to 50 L. Increase the speed to 18 rpm.
3. Continue the cell culture for a few more days until the cells reach about 2×10^6 cells/mL. Now add 50 L of fresh media. Increase the speed to 25 rpm and the angle to 7°. Monitor the oxygen levels in the culture carefully.
4. Continue the culture for a few more days, feeding the culture if desired, until cell viability begins to drop. Determine ahead of time the optimum viability at which to harvest.

For other size Cellbag bioreactors, adjust the volume proportionally. A Cellbag-100L would start at 5 L.

Comments

Do not rock too slowly. If growth appears poor, try raising the speed by 3 to 5 rpm and observe cell growth after 24 h. Ideally, check the dissolved oxygen online using a dissolved oxygen (DOOPT) system.

Refer to *Cellbag bioreactor pretreatment for the use of chemically defined lipid supplements* (28-9308-85 AA) for information on lipid pretreatment.

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Cultivation of T cells in suspension using WAVE Bioreactor Systems 2/10 and 20/50

Application

Cultivation of human T cells is a popular application for the WAVE Bioreactor™ system. Cell densities of up to 9×10^7 cells/mL at a very high viability have been achieved. Because Cellbag™ bioreactors are disposable, presterilized and completely contained, they are ideally suited to applications where the prevention of cross-contamination is critical. Additionally, the volume in a Cellbag™ bioreactor can be increased by a factor of 10, reducing inoculum requirements and the need for transfers. For example, a Cellbag-20L can be started with as little as 1 L of culture and fresh media can be added to match growth to bring the final batch volume to 10 L. Using the perfusion capabilities of the Cellbag bioreactor, very high cell densities can be generated while maintaining a closed system.

Inoculation

Inflate the Cellbag bioreactor with air and 10% CO_2 (if using bicarbonate buffered media) until it is rigid, add media and clamp the inlet and exhaust filters. Start rocking at 15 rocks per minute (rpm) and an angle of 6°. Allow the temperature and pH to equilibrate. Add cells to the Cellbag bioreactor. Initial cell densities should be based on media and supplement composition and the amount and type of mitogenic/growth factors added. Adjust the rpm and angle as described in "Culture parameters." Due to the efficient gas exchange that is possible with the WAVE Bioreactor system, the pH of bicarbonate buffered media can shift suddenly. Keeping the filters clamped during the early stages of the culture helps maintain a steady pH. Refer to "pH control" for some recommended techniques.

Operation

While the cells are growing, monitor cell density, viability, and metabolism adding media when required. If possible, add media that has been warmed to the operating temperature. Keep the Cellbag bioreactor rigidly inflated at all times. When using a System 2/10EH or 20/50EHT, make sure that there is good contact between the temperature probe and the culture. This will prevent overheating, which is particularly important if the culture is at a low volume. The oxygen levels of the culture are critical to successful cell growth. Monitor the oxygen levels with a dissolved oxygen (DOOPT) system, WAVEPOD™ Controller, or offline blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture. If perfusion is being used, it is important to begin while the cells are in the log growth phase. Start perfusing at a low rate initially (0.25 to 0.5 volumes/24 h). It is important to monitor nutrient and metabolite levels during perfusion and adjust the perfusion rate as needed.

Culture parameters

Rocking speed

The rocking speed is dependent on the culture volume, cell density, and Cellbag bioreactor size. For Cellbag-2L, -10L, and -20L set at 10 to 18 rpm initially. For very low volumes (10% to 20% of the working volume), an initial speed of 10 rpm is sufficient. Increase the speed to 20 to 25 rpm as more media is added to the culture. When at 100% of the Cellbag bioreactor working volume or at high cell density the speed might need to be as high as 25 rpm. For the Cellbag-50L, set the speed at 8 to 16 rpm initially. At 10% to 20% of the working volume, set the speed at 8 rpm. When at maximum volume, use a speed of 22 rpm. These are general guidelines only. Monitor the oxygen levels and adjust the rpm and rocking angle as needed.

Rocking angle

For the Cellbag-2L, -10L, and -20L an initial angle of 5° is sufficient. For Cellbag-50L use an initial angle of 4°. Generally, increase the angle as the oxygen demand increases. When the Cellbag bioreactor is at 100% of the working volume, an angle of 6° or 7° might be needed when using Cellbag-2L, -10L, or -20L. For the Cellbag-50L, an angle of 5° to 6° may be used when at maximum volume. Increase the rocking angle and decrease speed if excessive foaming is observed. Experience shows that this will help to break up the foam. It is important to monitor the oxygen levels of the culture and adjust the rocking angle as needed.

Aeration rate

The Cellbag bioreactor should be kept rigidly inflated. During inflation, a flow rate of up to 0.5 liters per minute (Lpm) can be used. Keep the filters clamped during the initial stages of the culture. Once vigorous growth is observed, set the flow rate to 0.1 Lpm for the Cellbag-2L, 0.2 Lpm for Cellbag-10L and Cellbag-20L, and 0.3 Lpm for Cellbag-50L during the run.

Temperature

The typical operating temperature for T cells is 36°C to 37°C.

pH control

pH control is critical. Due to the high gas transfer capacity of the WAVE Bioreactor system, pH can drift rapidly. Use the following procedure:

1. Initially, inflate the Cellbag bioreactor with 10% CO_2/air. After inflation, add media into the bioreactor and close off the inlet and outlet air filters. Rock at 15 rpm for 1 to 2 h to allow the pH and temperature to completely equilibrate. Before inoculation, check the pH by taking a sample. Adjust if necessary.
2. Inoculate with cells. Leave the inlet and outlet filters closed.
3. Monitor pH, glucose concentration, and cell density. Once the pH and glucose levels start dropping, switch to 5% CO_2/air with continuous airflow through the headspace. Once vigorous cell growth occurs, the media pH will not drift upwards and CO_2 concentration in the sweep gas can be used to control pH.
4. Increase the rocking speed and angle to maintain oxygen concentration. Use offline sampling, a DOOPT system, or WAVEPOD Controller to estimate dissolved oxygen concentration.

Scale up

A typical scale up for cells in a Cellbag-20L is given below. Keep in mind that this is a general guide only.

1. Fill 1 L of media into a Cellbag-20L. Add inoculum to give the appropriate cell density. Set the rocking speed to 10 rpm and the angle at 5°. Keep the system at operating temperature.
2. Continue to add media as the cells grow. Increase the rpm as the volume increases to maintain an appropriate dissolved oxygen level. At 4 L, the speed should be about 12 rpm. At 10 L, the speed can be as high as 25 rpm depending on cell density.
3. Once final volume in the Cellbag-20L has been reached, the culture can be grown until peak cell density is reached, then harvested. Greater cell densities can be achieved by perfusing the culture with fresh media. Try to keep the cells in the log growth phase when starting perfusion. Depending on cell growth rates, start the media flow at 0.25 to 0.5 culture volumes per day. For example, a culture at 10 L would need a perfusion rate of 2.5 to 5.0 L of fresh media per day to start. Maximum cell density might require 1.5 to 2.0 volumes of fresh media per day.

For other size Cellbag bioreactors, adjust the volume proportionally. A Cellbag-10L would start at 500 mL and Cellbag-2L at 100 mL. When using a Cellbag-50L with a working volume of 25 L, generally use a slightly reduced rpm and angle when compared to the settings used for smaller Cellbag bioreactors. For example use a maximum speed of 22 rpm and angle of 5° with a Cellbag-50L rather then the settings given above.

Comments

Generally, T cell derived cell lines have lower oxygen requirements then other mammalian cell lines. If the T cells do not grow well, try using a slower speed such as 6 rpm and a lower angle of 4° when at a 10% to 20% of the working volume of a Cellbag-20L. Make sure that the rpm and angle are sufficient to prevent the cells from settling out of the culture. Increase the rpm and angle as the volume of the culture increases.

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Cultivation of *E. coli* bacteria using WAVE Bioreactor Systems 2/10 and 20/50

Application

Although the WAVE Bioreactor™ system is designed for the cultivation of eukaryotic cells, it is possible to grow bacterial cultures in both the System 2/10EH and 20/50EHT with only slight modifications to the Cellbag™ bioreactor. Because the temperature control response in Cellbag bioreactors is rapid and precise, the temperature of the culture can be easily changed for induction purposes or to optimize product expression. The excellent oxygen transfer capability of the WAVE Bioreactor system allows high cell densities and good product expression. Also, because Cellbag bioreactors are disposable and supplied presterilized, set up and cleanup takes only minutes.

Inoculation

Because maximum rocks per minute (rpm) and angle are used during bacterial cultivation, fluid might build up in the Cellbag exhaust filter. Therefore, it is necessary to attach a foam trap on the outlet filter before installing the Cellbag bioreactor on the rocking platform.

Aseptically remove the exhaust filter by cutting the tie wrap and pulling the filter off the Cellbag bioreactor. Set the filter aside. Attach a length of autoclaved tubing to the filter port and connect the tubing to a sterile bottle. Attach the exhaust filter to an outlet on the bottle. Keep the check valve in place on the filter. Any foam will now be pushed into the bottle by the venting gas rather than into the filter. Install the modified Cellbag bioreactor on to the rocker platform and inflate until it is rigid. Use 30% oxygen and 70% nitrogen in the headspace to increase the dissolved oxygen. Fill the Cellbag bioreactor with growth media to the final volume desired and start rocking at about 15 rpm and an angle of 7°. Set the temperature controller to the desired temperature. Allow the temperature to equilibrate. Add the appropriate amount of log phase bacterial inoculum and increase the speed to 35 to 40 rpm and the angle to 10°.

Operation

The oxygen levels of the culture are critical to successful cell growth and production. For bacterial culture, oxygen is potentially the greatest limiting factor. Monitor the oxygen levels with a dissolved oxygen (DOOPT) system, WAVEPOD™ Controller, or offline blood gas analyzer. Maintain maximum rpm and angle to get the best oxygen transfer and use an oxygen-enriched gas source in the headspace. Monitor the foam trap. If too much liquid is being forced into the trap, it may be necessary to reduce the angle. Some loss of culture into the trap is expected. Use appropriate antifoam agents to reduce the amount of foam generated. Cell densities and protein yields should be comparable to shake flask cultures.

Culture parameters

Rocking speed and angle

For Cellbag-2L, -10L, and -20L maintain the speed at 35 to 40 rpm and the angle at 10°. For Cellbag-50L, set the speed to 33 to 38 rpm and the angle at 9°. Reduce the angle if an excessive amount of culture is being lost in the foam trap.

Aeration rate

The Cellbag bioreactor should be kept rigidly inflated. During inflation, a flow rate of up to 0.5 liters per minute (Lpm) can be used. Reduce the flow rate to around 0.1 Lpm for the Cellbag-2L, 0.2 Lpm for Cellbag-10L and Cellbag-20L, and 0.3 Lpm for Cellbag-50L during the run. Elevated levels of oxygen in the headspace are recommended. Use 30% oxygen and 70% nitrogen in the headspace. An O_2/Air Mix Plug-in Controller (O2MIX) or WAVEPOD Controller are available to provide precise oxygen levels in the headspace. Alternatively, a premixed cylinder of oxygen and nitrogen can be sourced from a laboratory gas supplier. Attach a source line to the air inlet of an airpump ensuring that that the supply pressure to the airpump does not exceed 1 to 3 psig. At a flow rate of 0.2 Lpm a standard cylinder should last approximately 1 month.

Temperature

The typical temperature for bacterial growth is 36°C to 37°C. Temperature adjustment might be required for induction of protein expression or to optimize expression.

Scale up

Because the inoculum volumes for bacterial culture are quite small, we recommend bringing the Cellbag bioreactor up to the maximum volume initially before adding the appropriate amount of bacterial culture. For a Cellbag-20L at 10 L working volume, the inoculation volume might range from 100 to 200 mL. Grow the cells until the desired density is reached, then add the induction agent. Adjust the temperature if required.

Comments

If cell growth is poor, try increasing the rocking speed. If product expression is poor, try increasing the rocking speed and aerate with oxygen-enriched air. In most cases these are symptoms of poor oxygen supply. Ideally, check the dissolved oxygen using a dissolved oxygen (DOOPT) system or WAVEPOD Controller and provide additional oxygen with the O_2/Air Mix Plug-in Controller.

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Cultivation of *E. coli* bacteria using WAVE Bioreactor System 200

Application

Although the WAVE Bioreactor™ System is designed for the cultivation of eukaryotic cells, it is possible to grow bacterial cultures with no special modifications of equipment and only slight modifications to the Cellbag™ bioreactor. Because the temperature control response in Cellbag bioreactors is rapid and precise, the temperature of the culture can be easily changed for induction purposes or to optimize product expression. The excellent oxygen transfer capability of the WAVE Bioreactor system allows high cell densities and good product expression. Also, because Cellbag bioreactors are disposable and supplied presterilized, set up and cleanup take only minutes.

Inoculation

Because maximum rocks per minute (rpm) and angle are used during bacterial cultivation, fluid might build up in the Cellbag exhaust filter. Therefore, it is necessary to attach a foam trap on the outlet filter before installing the Cellbag bioreactor on the rocking platform.

Place the Cellbag bioreactor in a laminar airflow hood. Aseptically remove the exhaust filter by cutting the tie wrap and pulling the filter off. Set the filter aside. Attach a length of autoclaved tubing to the filter port and connect the tubing to a sterile bottle. Attach the exhaust filter to an outlet on the bottle making sure that the tubing is long enough to reach outside of the System 200. Any foam will now be pushed into the bottle by the venting gas rather then into the filter. Install the modified Cellbag bioreactor onto the rocker platform and inflate until it is rigid. Fill the Cellbag to the final volume desired with growth media and start rocking at about 15 rpm and an angle of 7°. Set the temperature controller to the desired temperature. Allow the temperature to equilibrate. Add the appropriate amount of log phase bacterial inoculum and increase the speed to 25 rpm and the angle to 10°.

Operation

The oxygen levels of the culture are critical to successful cell growth and production. For bacterial culture, oxygen is potentially the greatest limiting factor. Monitor the oxygen levels with a dissolved oxygen (DOOPT) system or offline blood gas analyzer. Maintain maximum rpm and angle to get the best oxygen transfer and use an oxygen-enriched gas source in the headspace. Monitor the foam trap. If too much liquid is being forced into the trap, it might be necessary to reduce the angle. Some loss of culture into the trap is expected. Use appropriate antifoam agents to reduce the amount of foam generated. Cell densities and protein yields should be comparable to shake flask cultures.

Culture parameters

Rocking speed and angle

Maintain a speed of 25 rpm and angle of 10°, if possible, during the run. Reduce the angle if an excessive amount of culture is being lost in the foam trap.

Aeration rate

During inflation of the Cellbag bioreactor, a flow rate of up to 5 liters per minute (Lpm) can be used. The Cellbag bioreactor should be kept rigidly inflated. Reduce the flow rate to a minimum of 2 Lpm during the run. Flow rates of 2 to 3 Lpm are commonly used. Elevated levels of oxygen in the headspace are recommended. Use 30% oxygen and 70% nitrogen in the headspace. An O_2/Air Mix Plug-in Controller (O2MIX200) is available to provide precise oxygen levels in the headspace. Alternatively, a premixed cylinder of oxygen and nitrogen can be sourced from a laboratory gas supplier. At a flow rate of 2 Lpm, a standard cylinder should last approximately 3 days.

Temperature

The typical temperature for bacterial culture is 36°C to 37°C. Temperature adjustment might be required for induction or to optimize product expression..

Scale up

Because the inoculum volumes for bacterial culture are quite small, we recommend bringing the Cellbag bioreactor up to the maximum volume initially before adding the appropriate amount of bacterial culture. For a Cellbag-200L at 100 L working volume, the inoculation volume might range from 1000 to 2000 mL. Grow the cells until the desired density is reached, then add the induction agent. Adjust the temperature if required.

Comments

If cell growth is poor, try increasing the rocking speed. If product expression is poor, try increasing the rocking speed and aerate with oxygen-enriched air. In most cases these are symptoms of poor oxygen supply. Ideally, check the dissolved oxygen using a dissolved oxygen (DOOPT) system.

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Cultivation of BTI-TN-5B1-4 insect cells in suspension using WAVE Bioreactor Systems 2/10 and 20/50

Application

Cultivation of insect cells such as BTI-TN-5B1-4 (also known as High Five™ or T. ni) for the production of recombinant proteins using the baculovirus expression system is a popular application for the WAVE Bioreactor™ system. Cellbag™ disposable bioreactors can be used to expand cells for subsequent infection or for the infection itself. The excellent oxygen transfer capability of the WAVE Bioreactor system allows high cell densities and successful infections when the oxygen demand is greatest. Also, the volume in Cellbag bioreactors can be increased by a factor of 10, reducing inoculum requirements and the need for transfers. Insect cells can be seeded in the Cellbag bioreactor at a low volume, grown to a high density, then expanded to final volume for the infection. For example, a Cellbag-20L can be started with as little as 1 L of culture and fresh media can be added as the cells grow. Once the appropriate cell density is reached, the culture is brought to 10 L and the infection begun.

Inoculation

Inflate the Cellbag bioreactor with air until it is rigid. It is not necessary to add carbon dioxide to the headspace of insect cell cultures. Add media and start rocking at about 15 rocks per minute (rpm) and an angle of 7°. Allow the temperature to equilibrate. The temperature for insect cell culture is between 27°C and 28°C. Add cells to the Cellbag bioreactor. Cell inoculum should be sufficient to give a starting cell density of at least 0.5×10^6 cells/mL. Use a greater cell density if required by the cell line. Adjust the rpm and angle as described in “Culture parameters”.

Operation

While the cells are growing, monitor cell density, viability, and metabolism adding media when required. If possible, add media that has been warmed to operating temperature. If using a System 2/10EH or 20/50EHT, room temperature media or cooler media can be used. Keep the Cellbag bioreactor rigidly inflated at all times. The oxygen levels of the culture are critical to successful cell growth and production. Monitor the oxygen levels with a dissolved oxygen (DOOPT) system, WAVEPOD™ Controller, or offline blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture. Due to the high oxygen demand of baculovirus infections, it might be necessary to use 30% oxygen in the headspace rather then room air, especially if foaming becomes a problem and the maximum rpm and angle cannot be used. It is desirable to maintain at least 50% oxygen saturation in the culture during a baculovirus infection for the best protein expression. Segregate baculovirus activities from the stock insect cells during hood manipulations before infection to limit inadvertent baculovirus infections.

Culture parameters

Rocking speed

The rocking speed is dependent on the culture volume, cell density, and Cellbag bioreactor size. Generally, increase the rpm as the oxygen demand increases. For Cellbag-2L, -10L, and -20L set at 15 to 20 rpm initially. For very low volumes (10% to 20% of the working volume), an initial speed of 15 rpm is sufficient. Increase the speed to 20 to 25 rpm as more media is added to the culture. When at 100% of Cellbag bioreactor working volume or at high cell density, the speed might need to be as high as 25 rpm. For Cellbag-50L, set the speed at 12 to 18 rpm initially. At 10% to 20% of the working volume, set the speed at 12 rpm. When at maximum volume, use a speed of 22 rpm. These are general guidelines only. Monitor the oxygen levels and adjust the rpm and rocking angle as needed.

Rocking angle

For the Cellbag-2L, -10L, and -20L an initial angle of 6° is sufficient. For Cellbag-50L, use an initial angle of 5°. To increase oxygen transfer when the Cellbag is at 100% of the working volume, an angle of 8° to 9° might be needed when using Cellbag-2L, -10L, and -20L. For the Cellbag-50L, an angle of 7° to 8° may be used when at maximum volume. Keep in mind that BTI-TN-5B1-4 cells are larger and more shear-sensitive than other insect cell lines. When increasing the angle, observe the cells carefully for signs of stress. Increase the rocking angle and decrease speed if excessive foaming is observed. It is important to monitor the oxygen levels of the culture and adjust the rocking angle as needed.

Aeration rate

The Cellbag bioreactor should be kept rigidly inflated. During inflation, a flow rate of up to 0.5 liters per minute (Lpm) can be used. During the run, set the flow rate to 0.1 Lpm for the Cellbag-2L, 0.2 Lpm for Cellbag-10L and Cellbag-20L, and 0.3 Lpm for Cellbag-50L. Elevated levels of oxygen in the headspace might be needed, especially during the initial stages of the baculovirus infection. Use 30% oxygen and 70% nitrogen in the headspace. An O_2/Air Mix Plug-in Controller (O2MIX20) or WAVEPOD Controller are available to provide precise oxygen levels in the headspace during an infection. Alternatively, a premixed cylinder of oxygen and nitrogen can be sourced from a laboratory gas supplier. Attach a source line to the air inlet of an airpump ensuring that the supply pressure to the airpump does not exceed 1 to 3 psig. At a flow rate of 0.2 Lpm, a standard cylinder should last approximately 1 month.

Temperature

The typical operating temperature for insect cells is 27°C to 28°C.

Scale up

A typical scale up for a BTI-TN-5B1-4 insect cell infection in a Cellbag-20L is given below. Keep in mind that this is a general guide only. Different cell lines behave differently.

1. Fill 1 L of insect cell media into a Cellbag-20L. Add inoculum to give a starting cell count of at least 0.5×10^6 cells/mL. Set the rocking speed to 15 rpm and an angle of 6°. Keep the system at the operating temperature.
2. After 3 to 4 days of cultivation (depending on doubling rate), the cell count should reach over 4×10^6 cells/mL. At this stage, add 4 L of fresh media to bring the total volume to 5 L. Increase the speed to 20 rpm and the angle to 7°.
3. Continue the cell culture for a few more days until the cells reach about 4×10^6 cells/mL. Now add 5 L of fresh media along with concentrated virus (MOI of 0.5 to 1). Increase the speed to 25 rpm and the angle to 8°. Monitor the oxygen levels in the culture carefully at this point. Increase the oxygen levels to 30% in the headspace if needed.
4. Continue the culture for a few more days until cell viability begins to drop. Determine ahead of time the optimum viability at which to harvest.

For other size Cellbag bioreactors, adjust the volume proportionally. A Cellbag-10L would start at 500 mL and Cellbag-2L at 100 mL. When using a Cellbag-50L with a final working volume of 25 L, generally use a slightly reduced rpm and angle when compared to the settings used for smaller Cellbag bioreactors. For example use a maximum speed of 23 rpm and angle of 7° with a Cellbag-50L rather than the settings given above.

Comments

Do not rock too slowly. If growth appears poor, try raising the speed by 3 to 5 rpm and observe cell growth after 24 h. Ideally, check the dissolved oxygen using a dissolved oxygen (DOOPT) system or WAVEPOD Controller.

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Cultivation of BTI-TN-5B1-4 insect cells in suspension using WAVE Bioreactor System 200

Application

Cultivation of insect cells such as BTI-TN-5B1-4 (also known as High Five™ or T. ni) for the production of recombinant proteins using the baculovirus expression system is a popular application for the WAVE Bioreactor™ system. Cellbag™ disposable bioreactors can be used to expand cells for subsequent infection or for the infection itself. The excellent oxygen transfer capability of the WAVE Bioreactor system allows high cell densities and successful infections when the oxygen demand is greatest. Also, the volume in Cellbag bioreactors can be increased by a factor of 10, reducing inoculum requirements and the need for transfers. Insect cells can be seeded in the Cellbag bioreactor at a low volume, grown to a high density, then expanded to final volume for the infection. For example, a Cellbag-200L can be started with as little as 10 L of culture and fresh media can be added as the cells grow, once the appropriate cell density is reached, the culture is brought to 100 L and the infection begun.

Inoculation

Inflate the Cellbag bioreactor with air until it is rigid. It is not necessary to add carbon dioxide to the headspace of insect cell cultures. Set the airflow rate to a minimum of 2 liters per minute (Lpm). Add media and start rocking at about 15 rocks per minute (rpm) and an angle of 7°. Allow the temperature to equilibrate. The temperature for insect cell culture is between 27°C and 28°C. Add cells to the Cellbag bioreactor. Cell inoculum should be sufficient to give a starting cell density of at least 0.5×10^6 cells/mL. Use a greater cell density if required by the cell line. Adjust the rpm and angle as described in "Culture parameters."

Operation

While the cells are growing, monitor density, viability, and metabolism adding media when required. If possible, add media that has been warmed to room temperature. The WAVE Bioreactor System 200 will rapidly warm the culture to the operating temperature. Keep the Cellbag bioreactor rigidly inflated at all times. The oxygen levels of the culture are critical to successful cell growth and production. Monitor the oxygen levels with a dissolved oxygen (DOOPT) system or offline blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture. Due to the high oxygen demand of baculovirus infections, it might be necessary to use 30% oxygen in the headspace rather than room air, especially if foaming becomes a problem and maximum rpm and angle cannot be used. We recommend maintaining at least 50% oxygen saturation in the culture during a baculovirus infection for best protein expression. Segregate baculovirus activities from the stock insect cells during hood manipulations before infection to limit inadvertent baculovirus contamination.

Culture parameters

Rocking speed

The rocking speed is dependent on both the culture volume and cell density. Generally, increase the rpm as the oxygen demand increases. For very low volumes (10% to 20% of the working volume), an initial speed of 15 rpm is sufficient. Increase the speed to 20 to 25 rpm as more media is added to the culture. When at 100% of the Cellbag bioreactor working volume or at high cell density, the speed should be set at 25 rpm. These are general guidelines only. Monitor the oxygen levels and adjust the rpm and rocking angle as needed.

Rocking angle

An initial angle of 7º is sufficient. Because BTI-TN-5B1-4 insect cells tend to be shear-sensitive, try to maintain a low angle. When the Cellbag bioreactor is at 100% of the working volume increasing the angle to 8º or 9º can help increase oxygenation but monitor the cells carefully for signs of stress. Increase the rocking angle and decrease speed if excessive foaming is observed. Experience shows that this will help to break up the foam. It is important to monitor the oxygen levels of the culture and adjust the rocking angle as needed.

Aeration rate

During inflation of the Cellbag bioreactor, a flow rate of up to 5 Lpm can be used. The Cellbag bioreactor should be kept rigidly inflated. Reduce the flow rate to a minimum of 2 Lpm during the run. Airflow rates of 2 to 3 Lpm are commonly used. Elevated levels of oxygen in the headspace might be needed, especially during the initial stages of the baculovirus infection. Use 30% oxygen and 70% nitrogen in the headspace. An O_2/Air Mix Plug-in Controller (O2MIX200) is available to provide precise oxygen levels in the headspace during infections. Alternatively, a premixed cylinder may be sourced from a laboratory gas supplier. Attach a source line to the air inlet of an airpump ensuring that the supply pressure to the airpump does not exceed 1 to 3 psig. At a flow rate of 2 Lpm a standard cylinder should last 3 days.

Temperature:

The typical operating temperature for insect cells is 27°C to 28°C.

Scale up

A typical scale up for an insect cell infection in a Cellbag-200L is given below. Keep in mind that this is a general guide only. Different cell lines behave differently.

1. Fill 10 L of insect cell media into a Cellbag-200L. Add inoculum to give a starting cell count of at least 0.5×10^6 cells/mL. Set the rocking speed to 15 rpm and an angle of 7º. Keep the system at the operating temperature.
2. After 4 to 5 days of cultivation (depending on doubling rate), the cell count should reach over 4×10^6 cells/mL. At this stage, add 40 L of fresh media to bring the total volume to 50 L. Increase the speed to 20 rpm.
3. Continue the cell culture for a few more days until the cells reach about 4×10^6 cells/mL. Now add 50 L of fresh media along with concentrated virus (MOI of 0.5 to 1). Increase the speed to 25 rpm and keep the angle to 7º. Monitor the oxygen levels in the culture carefully at this point. If the oxygen levels drop below 50% in the culture, use a mix of 30% oxygen and 70% nitrogen in the headspace rather than room air.
4. Continue the culture for a few more days until cell viability begins to drop. Determine ahead of time the optimum viability at which to harvest.

Comments

If cell growth is poor, try increasing the rocking speed. If product expression is poor, try increasing the rocking speed and aeration with oxygen-enriched air. In most cases these are symptoms of poor oxygen supply. Ideally, check the dissolved oxygen online using a dissolved oxygen (DOOPT) system.

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Cultivation of Sf9 and Sf21 insect cells in suspension using WAVE Bioreactor Systems 2/10 and 20/50

Application

Cultivation of insect cells such as Sf9 and Sf21 for the production of recombinant proteins using the baculovirus expression system is a popular application for the WAVE Bioreactor™ system. Cellbag™ disposable bioreactors can be used to expand cells for subsequent infection or for the infection itself. In many ways Cellbag bioreactors are ideally suited for insect cell growth and baculovirus protein expression. The excellent oxygen transfer capability of the WAVE Bioreactor system allows high cell densities and successful infections when the oxygen demand is greatest. Furthermore, the volume in Cellbag bioreactors can be increased by a factor of 10, reducing inoculum requirements and the need for transfers. Insect cells can be seeded at a low volume, grown to a high density, then expanded to final volume for the infection. For example, a Cellbag-20L can be started with as little as 1 L of culture and fresh media can be added as the cells grow. Once the appropriate cell density is reached, the culture is brought to 10 L and infected.

Inoculation

Inflate the Cellbag bioreactor with air until it is rigid. It is not necessary to add carbon dioxide to the headspace of insect cell cultures. Add media and clamp the inlet and outlet filters. Start rocking at about 15 rocks per minute (rpm) and an angle of 7°. Allow the temperature to equilibrate. The temperature for insect cell culture is between 27°C and 28°C. Add cells to the Cellbag bioreactor. Cell inoculum should be sufficient to give a starting cell density of at least 0.5×10^6 cells/mL. Adjust the rpm and angle as described in "Culture parameters."

Operation

While the cells are growing monitor cell density, viability, and metabolism, adding media when required. If possible, add media that has been warmed to operating temperature. If using a System 2/10EH or 20/50EHT, room temperature media or cooler media can be used. The WAVE Bioreactor system will rapidly warm the culture to the appropriate temperature. Keep the Cellbag bioreactor rigidly inflated at all times. The oxygen levels of the culture are critical to successful cell growth and production. Monitor the oxygen levels with a dissolved oxygen (DOOPT) system, WAVEPOD™ Controller, or offline blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture. Due to the high oxygen demand of baculovirus infections, it might be necessary to use 30% oxygen in the headspace rather than room air, especially if foaming becomes a problem and maximum rpm and angle cannot be used. It is desirable to maintain at least 50% oxygen saturation in the culture during a baculovirus infection for best protein expression. Segregate baculovirus activities from the stock insect cells during hood manipulations before infection to limit inadvertent baculovirus infections.

Culture parameters

Rocking speed

The rocking speed is dependent on the culture volume, cell density, and Cellbag bioreactor size. Generally, increase the speed as the oxygen demand increases. For Cellbag-2L, -10L, and -20L set at 15 to 20 rpm initially. For very low volumes (10% to 20% of the Cellbag bioreactor working volume), an initial speed of 15 rpm is sufficient. Increase the speed to 20 to 25 rpm as more media is added to the culture. When at 100% of the working volume or at high cell density, the speed might need to be as high as 28 rpm.

For Cellbag-50L, set at 12 to 18 rpm initially. At 10% to 20% of the working volume, set the speed at 12 rpm. When at maximum volume, use a speed of 25 rpm. These are general guidelines only. Monitor the oxygen levels and adjust the rpm and rocking angle as needed.

Rocking angle

For Cellbag-2L, -10L, and -20L an initial angle of 7° is sufficient. For Cellbag-50L use an initial angle of 6°. To increase oxygen transfer when the Cellbag bioreactor is at 100% of its working volume, an angle of 9° or 10° might be needed when using Cellbag-2L, -10L, or -20L. For Cellbag-50L, an angle of 8° to 9° degrees may be used when at maximum volume. Increase the rocking angle and decrease

speed if excessive foaming is observed. Experience shows that this will help to break up the foam. It is important to monitor the oxygen levels of the culture and adjust the rocking angle as needed.

Aeration rate

The Cellbag bioreactor should be kept rigidly inflated. During inflation, a flow rate of up to 0.5 liters per minute (Lpm) can be used. During the run, set the flow rate to 0.1 Lpm for the Cellbag-2L, 0.2 to 0.3 Lpm for Cellbag-10L and Cellbag-20L, and 0.3 to 0.4 Lpm for Cellbag-50L. Elevated levels of oxygen in the headspace might be needed, especially during the initial stages of the baculovirus infection. Use 30% oxygen and 70% nitrogen in the headspace. An O_2/Air Mix Plug-in Controller (O2MIX20) or a WAVEPOD™ Controller is available to provide precise oxygen levels in the headspace during an infection. Alternatively, a premixed cylinder of oxygen and nitrogen can be sourced from a laboratory gas supplier. Attach a source line to the air inlet of an airpump ensuring that the supply pressure to the airpump does not exceed 1 to 3 psig. At a flow rate of 0.2 Lpm, a standard cylinder should last approximately 1 month.

Temperature

The typical operating temperature for insect cells is 27°C to 28°C.

Scale up

A typical scale up for an insect cell infection in a Cellbag-20L is given below. Keep in mind that this is a general guide only. Different cell lines behave differently. Some cells lines require inoculation densities of up to 1.5×10^6 cells/mL, but might yield higher cell densities at the end of culture. Before scaling up, determine the appropriate initial cell density as well as other growth characteristics of a particular cell line in a Cellbag bioreactor.

1. Fill 1 L of insect cell media into a Cellbag-20L. Add inoculum to give a starting cell count of at least 0.5×10^6 cells/mL. Set the rocking speed to 15 rpm and an angle of 7°. Keep the system at operating temperature.
2. After 2 to 3 days of cultivation (depending on doubling rate), the cell count should reach over 4×10^6 cells/mL. At this stage, add 4 L of fresh media to bring the total volume to 5 L. Increase the speed to 25 rpm and the angle to 8°.
3. Continue the cell culture for a few more days until the cells reach about 4×10^6 cells/mL. Now add 5 L of fresh media along with concentrated virus. MOI levels may vary depending upon application or preference. Increase the speed to 28 rpm and the angle to 9°. Monitor the oxygen levels in the culture carefully at this point. Increase the oxygen levels to 30% in the headspace if needed. Increase the rocking angle and decrease speed if excessive foaming is observed.
4. Continue the culture for a few more days until cell viability begins to drop. Determine ahead of time the optimum viability at which to harvest.

For other size Cellbag bioreactors, adjust the volume proportionally. A Cellbag-10L would start at 500 mL and Cellbag-2L at 100 mL. When using a Cellbag-50L with a working volume of 25 L, generally use a slightly reduced rpm and angle when compared to the settings used for smaller Cellbag bioreactors. For example use a maximum speed of 25 rpm and angle of 8° with a Cellbag-50L rather than the settings given above.

Comments

Do not rock too slowly. If growth appears poor, try raising the speed by 3 to 5 rpm and observe cell growth after 24 h. Ideally, check the dissolved oxygen using a dissolved oxygen (DOOPT) system or WAVEPOD Controller.

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Cultivation of Sf9 and Sf21 insect cells in suspension using WAVE Bioreactor System 200

Application

Cultivation of insect cell lines such as Sf9 and Sf21 for the production of recombinant proteins using the baculovirus expression system is a popular application for the WAVE Bioreactor™ system. Cellbag™ disposable bioreactors can be used to expand cells for subsequent infection or for the infection itself. The excellent oxygen transfer capability of the WAVE Bioreactor system allows high cell densities and successful infections when the oxygen demand is greatest. Also, the volume in Cellbag bioreactors can be increased by a factor of 10, reducing inoculum requirements and the need for transfers. Insect cells can be seeded in a Cellbag bioreactor at a low volume, grown to a high density, then expanded to final volume for the infection. For example, a Cellbag-200L can be started with as little as 10 L of culture and fresh media can be added as the cells grow. Once the appropriate cell density is reached, the culture is brought to 100 L and the infection begun.

Inoculation

Inflate the Cellbag bioreactor with air until it is rigid. It is not necessary to add carbon dioxide to the headspace of insect cell cultures. Set the airflow rate to 2 liters per minute (Lpm) minimum. Add media and start rocking at about 15 rocks per minute (rpm) and an angle of 7°. Allow the temperature to equilibrate. The temperature for insect cell culture is between 27°C and 28°C. Add cells to the Cellbag bioreactor. Cell inoculum should be sufficient to give a starting cell density of at least 0.5×10^6 cells/mL. Adjust the rpm and angle as described in "Culture parameters."

Operation

While the cells are growing, monitor cell density and metabolism adding media when required. If possible, add media that has been warmed to room temperature. Keep the Cellbag bioreactor rigidly inflated at all times. The oxygen levels of the culture are critical to successful cell growth and production. Monitor the oxygen levels with a dissolved oxygen (DOOPT) system or offline blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture. Due to the high oxygen demand of baculovirus infections, it might be necessary to use 30% oxygen in the headspace rather than room air, especially if foaming becomes a problem and maximum rpm and angle cannot be used. It is desirable to maintain at least 50% oxygen saturation in the culture during a baculovirus infection for best protein expression. Segregate baculovirus activities from the stock insect cells during hood manipulations before infection to limit inadvertent baculovirus infections.

Culture parameters

Rocking speed

The rocking speed is dependent on both the culture volume and cell density. Generally increase the rpm as the oxygen demand increases. For very low volumes (10% to 20% of the working volume), an initial speed of 15 rpm is sufficient. Increase the speed to 20 to 25 rpm as more media is added to the culture. When at 100% of the Cellbag bioreactor working volume or at high cell density, the speed should be set at 25 rpm. These are general guidelines only. Monitor the oxygen levels and adjust the rpm and rocking angle as needed.

Rocking angle

An initial angle of 7° is sufficient. To increase oxygen transfer when the Cellbag bioreactor is at 100% of the working volume, an angle of 9° to 10° might be needed. Increase the rocking angle and decrease speed if excessive foaming is observed. Experience shows that this will help to break up the foam. It is important to monitor the oxygen levels of the culture and adjust the rocking angle as needed.

Aeration rate

During inflation of the Cellbag bioreactor, a flow rate of up to 5 Lpm can be used. The Cellbag bioreactor should be kept rigidly inflated. Reduce the flow rate to 2 Lpm during the run. Airflow rates of 2 to 3 Lpm are commonly used. Elevated levels of oxygen in the headspace might be needed, especially during the initial stages of the baculovirus infection. Use 30% oxygen and 70% nitrogen in the headspace. An O_2/Air Mix Plug-in Controller (O2MIX200) is available to provide precise oxygen levels in the headspace during infections. Alternatively, a premixed cylinder of oxygen and nitrogen can be sourced from a laboratory gas supplier. Attach a source line to the air inlet of an airpump ensuring that the supply pressure to the airpump does not exceed 1 to 3 psig. At a flow rate of 0.2 Lpm, a standard cylinder should last approximately 1 month.

Temperature:

The typical operating temperature for insect cells is 27°C to 28°C.

Scale up

A typical scale up for an insect cell infection in a Cellbag-200L is given below. Keep in mind that this is a general guide only. Different cell lines behave differently. Before scaling up, determine the appropriate initial cell density as well as other growth characteristics of a particular cell line in a Cellbag bioreactor.

1. Fill 10 L of insect cell media into a Cellbag-200L. Add inoculum to give a starting cell count of at least 0.5×10^6 cells/mL. Set the rocking speed to 15 rpm and the angle at 7°. Keep the system at the operating temperature.
2. After 4 to 5 days of cultivation (depending on doubling rate), the cell count should reach over 4×10^6 cells/mL. At this stage, add 40 L of fresh media to bring the total volume to 50 L. Increase the speed to 20 rpm and the angle to 8°.
3. Continue the cell culture for a few more days until the cells reach about 4×10^6 cells/mL. Now add 50 L of fresh media along with concentrated virus (MOI of 0.5 to 1). Increase the speed to 25 rpm and the angle to 9°. Monitor the oxygen levels in the culture carefully at this point. If the oxygen levels drop below 50% in the culture, use a mix of 30% oxygen and 70% nitrogen in the headspace as described above.
4. Continue the culture for a few more days until cell viability begins to drop. Determine ahead of time the optimum viability at which to harvest.

Comments

If cell growth is poor, try increasing the rocking speed. If product expression is poor, try increasing the rocking speed and aeration with oxygen-enriched air. In most cases these are symptoms of poor oxygen supply. Ideally, check the dissolved oxygen online using a dissolved oxygen (DOOPT) system.

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Cultivation of plant cells in suspension using WAVE Bioreactor Systems 2/10 and 20/50

Application

Plant cell culture is increasingly being used as a powerful research tool for the investigation of plant physiology, the production of naturally occurring and recombinant compounds, and for clonal production of plant embryos for agriculture and industry. The excellent mixing and oxygenation capabilities of the WAVE Bioreactor™ system make it well suited to a variety of *in vitro* plant cell cultures. The low- shear environment allows for the growth of many cell, callus, and plant embryo types. Additionally, the lack of an internal impeller or sparger prevents clogging and tangling when growing hairy root cell culture.

Inoculation

The inoculation of the Cellbag™ disposable bioreactor is dependent on the type of plant cells to be cultivated. For individual plant cells, inflate with air until it is rigid, adding the required amount of growth media. Start rocking at 15 rocks per minute (rpm) and an angle of 6°. Allow the temperature to equilibrate. Add cells to a Cellbag bioreactor fitted with a screw cap port by gravity, peristaltic pump, or by pouring through the screw cap port. In the case of hairy root or callus cultivation, add the cells or plant fragments through the screw cap port of a Cellbag-2L, -10L, or -20L. Determine ahead of time the cell density required. Use a greater cell density if cell growth lags.

Operation

While the cells are growing, monitor cell density, viability, and metabolism adding media when required. Keep the Cellbag bioreactor rigidly inflated at all times to reduce foam formation. When using a System 2/10EH or 20/50EHT, make sure there is good contact between the temperature probe and the culture. This will prevent overheating, which is particularly important if the culture is at a low volume. The oxygen levels of the culture are critical to successful cell growth and production. Monitor the oxygen levels with a dissolved oxygen (DOOPT) system, WAVEPOD™ Controller, or offline blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture. Plant cells exhibit a variety of responses to oxygen concentration in the culture.

Culture parameters

Rocking speed

The rocking speed is dependent on the culture volume, cell density, and Cellbag bioreactor size. Generally, increase the rpm as the oxygen demand increases. For Cellbag-2L, -10L, and -20L set at 12 to 20 rpm initially. For very low volumes (10% to 20% of the working volume), an initial speed of 12 rpm is sufficient. Increase the speed to 20 to 25 rpm as more media is added to the culture. When at 100% of Cellbag bioreactor working volume or at high cell density the speed might need to be as high as 25 rpm. For Cellbag-50L, set the speed at 10 to 18 rpm initially. At 10% to 20 % of the working volume, set the speed at 10 rpm. When at maximum volume use a speed of 22 rpm. These are general guidelines only. Monitor the oxygen levels and adjust the rpm and rocking angle as needed.

Rocking angle

For the Cellbag-2L, -10L, and -20L an initial angle of 6° is sufficient. For Cellbag-50L, use an initial angle of 5°. To increase oxygen transfer when the Cellbag bioreactor is at 100% of the working volume, an angle of 7° or 8° might be needed when using a Cellbag-2L, -10L, or -20L. For the Cellbag-50L, an angle of 6° to 7° may be used when at maximum volume. Increase the rocking angle and decrease speed if excessive foaming is observed. Experience shows that this will help to break up the foam. It is important to monitor the oxygen levels of the culture and adjust the rocking angle as needed.

Aeration rate

The Cellbag bioreactor should be kept rigidly inflated. During inflation, a flow rate of up to 0.5 liters per minute (Lpm) can be used. During the run, set the flow rate to 0.1 Lpm for the Cellbag-2L, 0.2 Lpm for Cellbag-10L and Cellbag-20L, and 0.3 Lpm for Cellbag-50L.

Temperature

The typical operating temperature for plant cells is 25°C.

Scale up

A typical scale up for hairy root plant cells in a Cellbag-20L is given below. Keep in mind that this is a general guide only. Different cell lines behave differently.

1. Aseptically, add the hairy root starter cultures through the screw cap port. Add 5 L of growth media. Start at a rocking speed of 10 rpm and an angle of 5º. Keep the system at the operating temperature. Use growth lights on a 16 h on and 8 h off schedule.
2. After 2 to 3 days of cultivation, add 5 L of fresh media to bring the total volume to 10 L. Increase the speed to 15 rpm.
3. Continue to monitor the oxygen levels in the culture and increase the rpm and angle as required. After 8 to 10 days, the speed should be set at 25 rpm and the angle at 8º.
4. Continue the culture by drawing off up to 90% of the growth media and refeeding with fresh media. Continue to monitor the oxygen levels and adjust the rpm and angle to increase the oxygen if required. Determine ahead of time the optimum time at which to harvest.

For other size Cellbag bioreactors, adjust the volume proportionally. A Cellbag-10L would start at 2.5 L and Cellbag-2L at 500 mL. When using a Cellbag-50L with a working volume of 25 L, generally use a slightly reduced rpm and angle when compared to the settings used for smaller Cellbag bioreactors. For example, use a speed of 23 rpm and angle of 6º with a Cellbag-50L rather than the settings given above.

Comments

Do not rock too slowly. If growth appears poor, try raising the rocking speed by 3 to 5 rpm and observe cell growth after 24 h. Ideally, check the dissolved oxygen using a dissolved oxygen (DOOPT) system or WAVEPOD Controller.

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Cultivation of yeast cells using WAVE Bioreactor Systems 2/10 and 20/50

Application

Although the WAVE Bioreactor™ Systems 2/10 and 20/50 are designed for the cultivation of mammalian or insect cells, their characteristics allow for the growth of yeast cultures with no modifications of equipment. The excellent oxygen transfer capability of the WAVE Bioreactor system allows high cell densities and good product expression. Additionally, the volume in the Cellbag™ bioreactor can be increased by a factor of 10, reducing inoculum requirements and the need for transfers. For example, a Cellbag-20L can be started with as little as 1 L of culture and fresh media can be added to match growth to bring the final batch volume to 10 L. Since Cellbag bioreactors are presterilized and disposable, set up and cleanup takes only minutes.

One of the most common types of yeast cultivated for recombinant protein production is *Pichia pastoris*. In the expression system described here, the protein of interest is produced without induction. In many *Pichia* strains, the protein of interest is produced with methanol induction. A side-effect of the use of methanol is the generation of significant amounts of heat. For methanol- induced protein production, the WAVE Bioreactor system may be placed in a refrigerated environment to prevent overheating.

Inoculation

Inflate the Cellbag bioreactor with air until it is rigid. It is not necessary to add carbon dioxide to the headspace of yeast cell cultures. Add media and start rocking at about 15 rocks per minute (rpm) and an angle of 7°. Allow the temperature to equilibrate. The temperature for yeast culture is 30°C. Temperatures over 32°C can limit protein expression. Add yeast inoculum to the Cellbag bioreactor.

Operation

While the cells are growing, continue to monitor cell density by optical density and the dissolved oxygen levels in the culture with a dissolved oxygen (DOOPT) system, WAVEPOD™ Controller, or offline blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture. Due to the high oxygen demand of yeast cultures, it might be necessary to use 30% oxygen in the headspace rather than room air, especially if foaming becomes a problem and maximum rpm and angle cannot be used. It is desirable to maintain at least 20% oxygen saturation in the culture for best protein expression.

A fed-batch method is very effective in achieving high cell densities and product yields. In a fed-batch system, it is important to limit the initial volume of the batch to about 50% to70% of the maximum volume to leave room for feeding. Typical feeds are made of 50% glycerol with trace elements and minerals. A continuous feeding strategy is most efficient. A typical feed rate would be 0.5 mL/min/L of culture. Try to anticipate the oxygen demands of the culture by gradually increasing the angle, rpm, and oxygen in the headspace during the feeding process. If the dissolved oxygen falls below 20% during the feed, slow or interrupt the feed until the oxygen levels spike. At that point, adjust the rpm, angle, or amount of oxygen in the headspace to increase the dissolved oxygen in the culture.

Culture parameters

Rocking speed

The rocking speed is dependent on the culture volume, cell density, and Cellbag bioreactor size. For Cellbag-2L, -10L, and -20L set at 20 to 25 rpm initially. For very low volumes (10% to 20% of the working volume), an initial speed of 20 rpm is sufficient. Increase the speed to 25 to 30 rpm as more media is added to the culture. When at 100% of the Cellbag bioreactor working volume or at high cell density, the speed might need to be as high as 30 rpm. For a Cellbag-50L, set the speed at 18 to 23 rpm initially. At 10% to 20% of the working volume, set the speed at 18 rpm. When at maximum volume, use a speed of 28 rpm. These are general guidelines only. Monitor the oxygen levels and adjust as needed.

Rocking angle

For the Cellbag-2L, -10L, and -20L an initial angle of 7° is sufficient. For Cellbag-50L, use an initial angle of 6°. When the Cellbag bioreactor is at 100% of its working volume, an angle of 9° or 10° might be needed when using Cellbag-2L, -10L, or 20L. For the Cellbag-50L ,an angle of 8° to 9° may be used when at maximum volume. Increase the rocking angle and decrease speed if excessive foaming is observed.

Experience shows that this will help to break up the foam. It is important to monitor the oxygen levels of the culture and adjust the rocking angle as needed.

Aeration rate

The Cellbag bioreactor should be kept rigidly inflated. During inflation, a flow rate of up to 0.5 liters per minute (Lpm) can be used. During the run, set the flow rate to 0.1 Lpm for the Cellbag-2L, 0.2 to 0.3 Lpm for Cellbag-10L and Cellbag-20L, and 0.3 to 0.4 Lpm for Cellbag-50L. Elevated levels of oxygen in the headspace might be needed. Use 30% oxygen and 70% nitrogen in the headspace. An O2MIX20 module or a WAVEPOD Controller are available to provide precise oxygen levels in the headspace during an infection. Alternatively, a premixed cylinder of oxygen and nitrogen can be sourced from a laboratory gas supplier by requesting a calibration grade mixture. Attach a source line to the air inlet of an airpump ensuring that the supply pressure to the airpump does not exceed 1 to 3 psig. At a flow rate of 0.2 Lpm, a standard cylinder should last approximately 1 month.

Temperature

Typical operating temperature for yeast cells is 30°C.

pH control

Yeast cultures tend to stabilize between pH 3 and 5. Depending on the characteristics of the protein of interest and proteolytic activity, it might be necessary to increase the pH. This can be achieved by adding sodium or ammonium hydroxide. A pH Probe and pH20 or a WAVEPOD Controller are available to control the addition of a base to the culture.

Scale up

A typical scale up for a *Pichia* culture in a Cellbag-20L is given below. Keep in mind that this is a general guide only. Different cell lines behave differently.

1. Fill 1 L of yeast growth media into a Cellbag-20L. The amount of inoculum should be between 10% and 20% of the initial volume. Set the rocking speed to 15 rpm and an angle of 7º. Keep the system at the operating temperature.
2. After 12 to 24 h of cultivation, add 4 L of fresh media to bring the total volume to 5 L. Increase the speed to 25 and the angle to 8º. Monitor the cell numbers by optical density. Use a dissolved oxygen (DOOPT) system, WAVEPOD Controller, or offline analyzer to measure the dissolved oxygen.
3. Continue the cell culture for 12 h until the dissolved oxygen begins to spike. An increase in the dissolved oxygen indicates a lack of metabolic activity. Begin the feed at 2.5 mL/min. Monitor the oxygen levels in the culture carefully at this point. Increase the speed to 28 rpm. Increase the oxygen levels to 30% in the headspace if needed. Increase the angle if excessive foaming is observed.
4. Continue the culture for another 40 h until maximum volume is reached.

Adjust the parameters given above when using a Cellbag bioreactor of a different size.

Comments

The feed rate and length of the culture are important in optimizing protein expression. Depending on the characteristics of the protein of interest, it might be useful to either extend the life of the culture by slowing the feed rate or increase the feed rate to shorten the culture time. Temperature can also be adjusted for protein expression and stability in the culture.

The use of methanol induction in yeast culture generates significant amounts of heat. The Cellbag bioreactor is not able to dissipate this heat unless the environment is significantly cooler than room temperature. A refrigerated environment is recommended.

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WAVE
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Cultivation of yeast cells using WAVE Bioreactor System 200

Application

Although the WAVE Bioreactor™ system is designed for the cultivation of eukaryotic cells, it is possible to grow yeast cultures with no modifications to the equipment. The excellent oxygen transfer capability of the WAVE Bioreactor system allows high cell densities and good product expression. Furthermore, the volume in Cellbag™ bioreactors can be increased by a factor of 10, reducing inoculum requirements and the need for transfers. For example, a Cellbag-200L can be started with as little as 10 L of culture and fresh media can be added to match growth to bring the final batch volume to 100 L. Also, because Cellbag bioreactors are disposable and supplied presterilized, set up and cleanup takes only minutes.

One of the most common types of yeast cultivated for recombinant protein production is *Pichia pastoris*. This procedure refers to the growth of *P. pastoris* in a WAVE Bioreactor System 200. In the expression system described, the protein of interest is produced without induction. In many *Pichia* strains the protein of interest is produced with methanol induction. A side effect of the use of methanol is the generation of significant amounts of heat. For methanol induced protein production, the WAVE Bioreactor system may be placed in a refrigerated environment to prevent overheating.

Inoculation

Inflate the Cellbag bioreactor with air until it is rigid. It is not necessary to add carbon dioxide to the headspace of yeast cell cultures. Add media and start rocking at about 15 rocks per minute (rpm) and an angle of 7°. Allow the temperature to equilibrate. The temperature for yeast culture is 30°C. Temperatures over 32°C may limit protein expression. Add yeast inoculum to the Cellbag bioreactor.

Operation

While the cells are growing, monitor cell density by optical density and the dissolved oxygen levels in the culture with a dissolved oxygen (DOOPT) system or offline blood gas analyzer. Adjust the rpm and angle in response to the oxygen demands of the culture. Due to the high oxygen demand of yeast cultures, it might be necessary to use 30% oxygen in the headspace rather then room air, especially if foaming becomes a problem and maximum rpm and angle cannot be used. We recommend maintaining at least 20% oxygen saturation in the culture for best protein expression.

A fed-batch method is very effective in achieving high cell densities and product yields. In a fed-batch system, it is important to limit the initial volume of the batch to 50% to 70% of the maximum working volume to leave room for feeding. Typical feeds are made of 50% glycerol with trace elements and minerals. A continuous feeding strategy is most efficient. A typical feed rate would be 0.5 mL/min/L of culture. Try to anticipate the oxygen demands of the culture by gradually increasing the angle, rpm and oxygen in the headspace during the feeding process. If the dissolved oxygen falls below 20% during the feed, slow or interrupt the feed until the oxygen levels spike. At that point adjust the rpm, angle, or amount of oxygen in the headspace to increase the dissolved oxygen in the culture.

Culture parameters

Rocking speed

The rocking speed is dependent on both the culture volume and cell density. For very low volumes (10% to 20% of the working volume), an initial speed of 15 rpm is sufficient. Increase the speed to 20 to 25 rpm as more media is added to the culture. When at 100% of the Cellbag bioreactor working volume or at high cell density, set the speed at 25 rpm. These are general guidelines only. Monitor the oxygen levels and adjust as needed.

Rocking angle

An initial angle of 7° is sufficient. When the Cellbag bioreactor is at 100% of the working volume, an angle of 9° to 10° might be needed. Increase the rocking angle and decrease speed if excessive foaming is observed. Experience shows that this will help to break up the foam. It is important to monitor the oxygen levels of the culture and adjust the angle as needed.

Aeration rate

During inflation of the Cellbag bioreactor, a flow rate of up to 5 liters per minute (Lpm) can be used. The Cellbag bioreactor should be kept rigidly inflated. Reduce the flow rate to 2 Lpm during the run. Airflow rates of 2 to 3 Lpm are commonly used. Elevated levels of oxygen in the headspace might be needed. Use 30% oxygen and 70% nitrogen in the headspace. An O_2/Air Mix Plug-in Controller (O2MIX200) is available to provide precise oxygen levels in the headspace during an infection. Alternatively, a premixed cylinder of oxygen and nitrogen may be sourced from a laboratory gas supplier. Attach a source line to the air inlet of an airpump ensuring that the supply pressure to the airpump does not exceed 1 to 3 psig. At a flow rate of 2 Lpm, a standard cylinder should last approximately 3 days.

Temperature

The typical operating temperature for yeast cells is 30°C.

pH control

The pH of yeast cultures stabilizes between pH 3 and 5. Depending on the characteristics of the protein of interest and proteolytic activity, it might be necessary to increase the pH. This can be achieved by adding sodium or ammonium hydroxide. A pH probe and pH200 Controller to control the addition of a base to the culture are available.

Scale up

A typical scale up for a *P. pastoris* culture in a Cellbag-200L is given below. Keep in mind that this is a general guide only. Different cell lines behave differently.

1. Fill 10 L of yeast growth media into a Cellbag-200L. The amount of inoculum should be between 10% and 20% of the initial volume. Set the rocking speed to 15 rpm and angle at 7º. Keep the system at the operating temperature.
2. After 12 to 24 h of cultivation, add 40 L of fresh media to bring the total volume to 50 L. Increase the speed to 20 rpm and the angle to 8º. Monitor the cell density by optical density. Use a dissolved oxygen (DOOPT) system or offline analyzer to measure the dissolved oxygen.
3. Continue the cell culture for 12 h until the dissolved oxygen begins to spike. An increase in the dissolved oxygen indicates a lack of metabolic activity. Begin the feed at 25 mL/min. Monitor the oxygen levels in the culture carefully at this point. Increase the speed to 28 rpm. Increase the oxygen levels to 30% in the headspace if needed.
4. Continue the culture for another 40 h until maximum volume is reached.

Adjust the parameters given above when using a different size Cellbag bioreactor.

Comments

The feed rate and length of the culture are critical in optimizing protein expression. Depending on the characteristics of the protein of interest, it might be useful to extend the life of the culture by slowing the feed rate or increasing the feed rate to shorten the culture time. Temperature can also be adjusted for protein expression and stability in the culture.

The use of methanol induction in yeast culture generates significant amounts of heat. The Cellbag bioreactor is not able to dissipate this heat unless the environment is significantly cooler than room temperature. A refrigerated area is recommended.

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