Simplified and reliable protein production from an insect cell baculovirus expression vector system (BEVS) using single-use ReadyToProcess WAVE 25 bioreactor system

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CY13643-21May20-AN

Simplified and reliable protein production from an insect cell baculovirus expression vector system (BEVS) using single-use ReadyToProcess WAVE™ 25 bioreactor system

For applications such as research studies and preclinical trials, quick production of small amounts of recombinant proteins is often required. Protein production in insect cells using the baculovirus expression vector system (BEVS) is commonly used for such applications, as insect cells are capable of most post-translational modifications required for a fully functional and soluble product and the genetic engineering steps are straightforward. In this application note, we describe how ReadyToProcess WAVE 25 bioreactor system can simplify operations in insect cell applications, while providing stable culture conditions though accurate process control. This single-use system is easy to use and can minimize hands-on time compared with culturing in shaker flasks. Further, the enhanced control capabilities allow for a repeatable process outcome with reliable data to support conclusions for future decision making.

Introduction

BEVS is widely used for production of baculovirus particles and recombinant proteins in insect cells (1, 2). Production is fast and easy and enables high yields, efficient protein secretion, and a eukaryotic glycan structure (3). Furthermore, the BEVS offers the possibility of coexpression of several proteins, allowing production of multisubunit proteins such as transcription factor complexes or virus-like particles (4-9), which makes the system well-suited for many applications in basic research, biotechnology, and in the biopharmaceutical industry. Insect cell lines commonly used together with the BEVS technology are derived from *Spodoptera frugiperda* (Sf9, Sf21) and *Trichoplusia ni* (High Five™). Cultivation is typically conducted at temperatures of 27°C to 28°C using air or oxygen enriched air to provide oxygen to the culture. Carbon dioxide is typically not needed.

This application note demonstrates the suitability of ReadyToProcess WAVE 25 bioreactor system for use in recombinant protein expression using the BEVS in an insect cell line. The bioreactor system allows reliable monitoring and control of culture parameters, including accurate temperature control at 27°C to 28°C without temperature overshoot during warm-up. With the use of ReadyToProcess™ Pump 25, the cultures can be fed with a predefined amount of cell culture medium at a specific time point, thereby minimizing the overall hands-on time required and facilitating addition of fresh medium during critical culture steps. For shaker flasks, several flasks are typically required when the culture volume increases above 1000 mL. Compared with culturing in five flasks at 1000 mL each, the process becomes simpler and more controlled when culturing in one 10 L Cellbag™ bioreactor (5 L working volume) using ReadyToProcess WAVE 25. With the bioreactor system, manual operation such as pipetting is minimized and the high containment enables work in open laboratory space without the need for a safety cabinet.

Here, we describe the expression of $(histidine)_6$-tagged enhanced green fluorescent protein (EGFP) using the BEVS in Sf21 cells cultured in the single-use ReadyToProcess WAVE 25 system. Relative expression levels were determined by Western blotting using the accurate and standardized Amersham™ WB system.

Materials and methods

Virus amplification

Sf21 cells (Invitrogen) were used for propagation of baculovirus containing the gene for N-terminally $(histidine)_6$-tagged EGFP (GenScript) in Sf900III SFM growth medium (Invitrogen). Cells (0.5×10^6 cells/mL) were infected at a multiplicity of infection (MOI) of 0.02. Infected cells were incubated at 27°C, 100 rpm for 7 days before harvested by centrifugation at 2500 × g for 15 min. For storage, the virus stock solution was supplemented with 5% fetal bovine serum and kept in dark at 4°C until use.

Shaker flask cultures

Sf21 cells were propagated in 250 mL shaker flask cultures using Sf900III SFM as growth medium. At day 4, cells were harvested by gentle centrifugation at 150 × g for 5 min in room temperature (RT) and resuspended in 20 mL virus stock solution in centrifuge tubes. The suspensions with cells and viruses were incubated for 30 min at 27° C in a shaker incubator. After incubation, the suspensions were diluted to 100 mL (2×10^6 cells/mL) with EX-CELL™ 420 production medium (Sigma-Aldrich) and transferred to 500 mL shaker flasks. The shaker flasks were incubated for five days in a shaker incubator at 27°C and 100 rpm. Cultures were performed in duplicate.

For determination of cell growth and viability, 1.5 mL samples were taken on day 0, 5, and 7 post infection.

Bioreactor cultures

For setting up the ReadyToProcess WAVE 25 system, pH and DO sensors were connected to a 10 L Cellbag bioreactor culture chamber. The culture chamber was attached to the tray on the rocker base unit. Gas tubing was connected from gas/pressure air sources to the CBCU control unit and from the control unit to the culture chamber. From the UNICORN™ system control software, ***Start a new run*** was selected and Cellbag size, feed flask pump tubing inner diameter, and pH and DO values from the Cellbag bioreactor were entered. Selected culture parameters are listed in Table 1.

Using ReadyToProcess Pump 25, 1000 mL Sf900III SFM growth medium was added to the culture chamber. Gas flow (100% air), rocking, and temperature control were started. The pH and DO sensors were allowed to soak and equilibrate for 2 to 4 h. When a medium temperature of 27°C was achieved, a 2 mL sample was taken for pH measurement to determine the accuracy in comparison with a reference pH instrument. The DO sensor was calibrated to 100% air saturation. DO control was performed according to Table 1. To test for sterility, the temperature control was turned off and the system was run over night at the selected rocking and gas flow settings.

Sf21 cells were propagated in 1000 mL shaker flasks. At time of inoculation, Sf21 cells were seeded to 0.5×10^6 cells/mL in a total volume of 1500 mL Sf900III SFM growth medium. At a cell density of 3 to 4 × 10^6 cells/mL (after 3–4 d), 1000 mL virus stock solution was added. The culture was incubated for 1 h before 2500 mL fresh EX-CELL 420 production medium was added to a final working volume of 5000 mL. Runs were performed in duplicate.

Samples of 1.5 mL were collected daily and measured for cell growth, viability, and metabolites. At day 3, 6, and 10 post-infection, cells were centrifuged at 150 × g for 5 min at RT and the supernatant were stored at -20°C until expression analysis.

Table 1. Culture parameters for the ReadyToProcess WAVE 25 production runs

Agitation rate	27 rpm
Angle	5°
Temperature	27°C
Dissolved oxygen (DO)	50%
DO control strategy using O_2	Gas flow rate > 0.25 L/min (> 0.02 vvm*). Oxygen on demand.
Target inoculation cell concentration	0.5×10^6/mL
Target cell concentration at time of infection	$3–4 \times 10^6$/mL
Target cell concentration post infection	$1–2 \times 10^6$/mL
pH	Not controlled

* Volumes of air per volume of medium per minute.

Determination of cell growth, viability, and metabolite content

Cell growth, viability, and metabolite content were determined using a BioProfile Flex™ analyzer (Nova Biomedical).

Determination of protein expression

Relative expression levels of $(histidine)_6$-tagged EGFP were determined by SDS-PAGE and Western blotting (10 µL sample/lane) using the Amersham WB system. Mouse anti-$(histidine)_6$ monoclonal IgG1 antibody (2 ng/µL in phosphate buffered saline containing 0.05% Tween™) was used as primary antibody and Cy™3B-conjugated goat-anti-mouse polyclonal antibody (0.4 ng/µL in phosphate buffered saline containing 0.05% Tween) was used as secondary antibody. Signal intensities for target proteins on the membrane image were compared and the relative expression levels determined.

Results and discussion

Cell growth and viability

Cell growth and viability in the shaker flask cultures are shown in Table 2. At day 0, cells were infected and a complete change of medium to production medium was made. Immediately after infection, cell density and viability started to decrease.

Table 2. Cell growth and viability in duplicate shaker flask cultures

Culture time (d) from infection	Cell density (viable cells/mL)		Cell viability (%)	
	Flask 1	Flask 2	Flask 1	Flask 2
0	2.26×10^6	2.26×10^6	98.3	98.3
5	1.31×10^6	1.32×10^6	59.3	62.9
7	1.56×10^6	1.49×10^6	56.9	54.8

For the bioreactor cultures, cell growth and viability are shown in Figure 1. Cells exhibited high viability and growth increased steadily until day 4 when cells were infected. Upon infection, production medium up to a final working volume of 5 L was added to the culture.

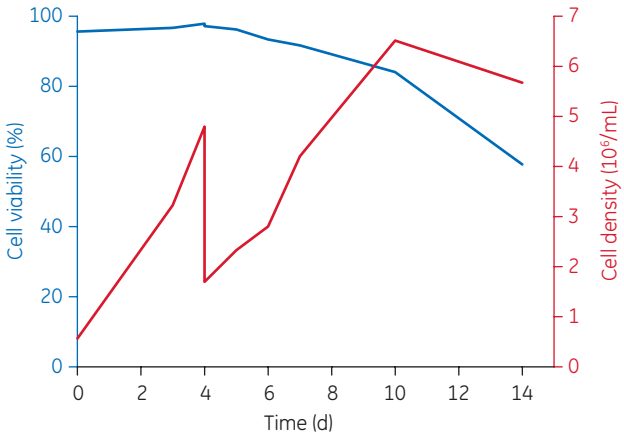

Fig 1. Cell growth and viability for the duplicate ReadyToProcess WAVE 25 runs. Cells were infected at day 4 and harvested at day 14.

Recombinant protein expression

Expression of $(histidine)_6$-tagged EGFP was analyzed by Western blotting using the Amersham WB system (Fig 2). Amersham WB allows for accurate quantitation and provides reproducible results using a standardized Western blotting workflow that is fully integrated and optimized for hardware, software, consumables, and reagents.

For the bioreactor cultures, samples were taken on day 3, 6, and 10 post-infection. Good expression was observed in the bioreactor samples from day 3 post-infection in the duplicate cultures, run 1 and 2. Although the highest expression was measured at day 10 post-infection, there was no significant increase in expression after day 6 post-infection.

The expression levels might have been further improved and the production time shortened if the added volume of fresh production medium would have been larger. A medium exchange prior to infection had in preliminary experiments shown to be crucial for achieving a good recombinant protein expression. However, the large volume of the virus stock solution used in this study made it unfeasible to add more medium.

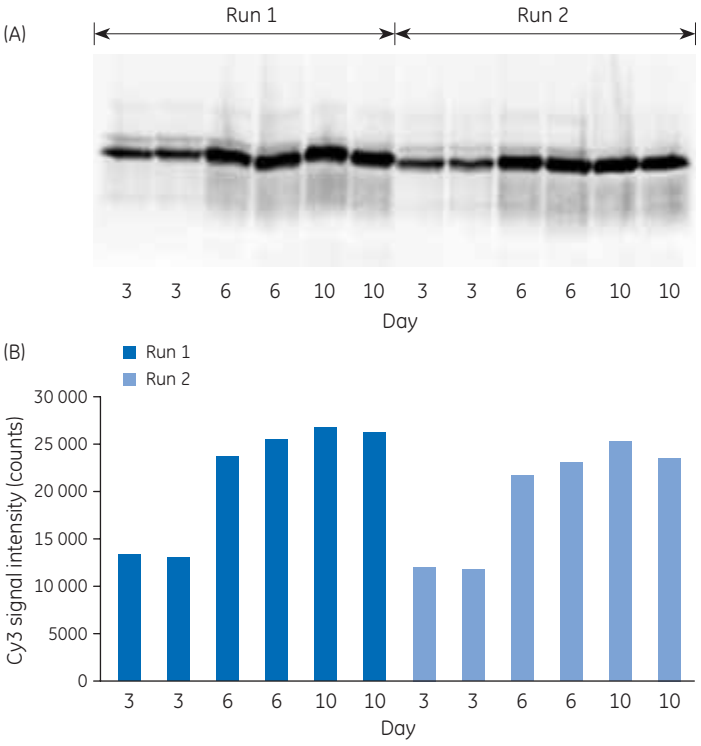

Fig 2. Expression of $(histidine)_6$-tagged EGFP. (A) Western blotting membrane image of samples from the ReadyToProcess WAVE 25 cultures. (B) Corresponding Cy3 fluorescence intensity in samples from bioreactor cultures. All samples were analyzed in duplicate.

Monitoring and control of process parameters

For stable culture conditions, ReadyToProcess WAVE 25 automatically monitors and controls process parameters.

As shown in the process picture from one of the bioreactor cultures in Figure 3, the weight was stable throughout the process. The medium addition day 4 was also accurately controlled. The dip in weight at day 4 was due to an intermittent removal of the lid and the occasional small spikes were caused by manual interactions during sampling.

The oxygen consumption of the culture increased during the initial phase of the infection period (day 4 to 6 corresponds to 0 or 2 days post-infection) and DO decreased until day 8. Thereafter, DO increased gradually again until the end of the culture. This oxygen consumption pattern most likely reflects the overall metabolic state of the culture, with an increased metabolic activity during the time when most of the recombinant protein is expressed. The pH was not controlled in the bioreactor cultures but remained stable at 6.19 to 6.21.

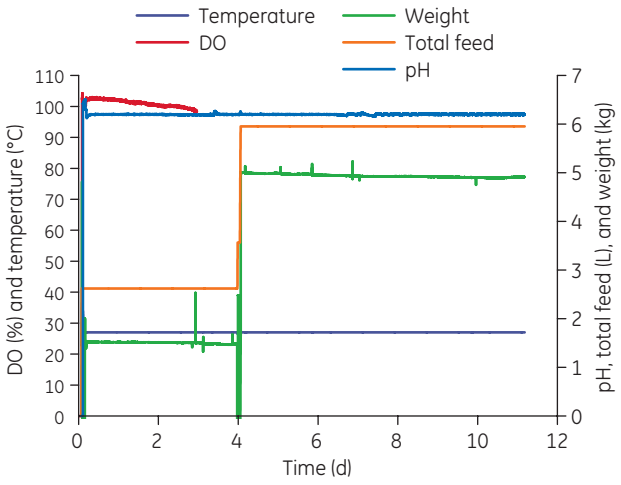

Fig 3. Process picture extracted from UNICORN 6.4 displaying temperature, DO, weight, total feed, and pH during the course of the culture.

Conclusion

ReadyToProcess WAVE 25 bioreactor system can simplify operations in small-scale applications, while providing stable culture conditions though accurate process control. In this application note, the system was used for culturing of Sf21 cells using the BEVS to produce recombinant $(histidine)_6$-tagged EGFP. The culture conditions were stable and cell growth, protein expression, and culture parameters were similar between two duplicate bioreactor cultures.

The temperature was stable at the set point 27°C, and the DO level remained above 40% without the need for oxygen enrichment. The ReadyToProcess WAVE 25 bioreactor system meets the requirements of processes including insect cell expression systems, providing a fast and easy way to reliable protein production in bench-top bioreactor scale. The Amersham WB system offered a convenient solution to accurate monitoring of protein expression levels over the culture period.

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29-1349-15 AA 11/2014