HyClone media and supplements

Reconstitution protocol

ActiCHO P Powder media

This protocol provides instructions for reconstituting ActiCHO™ P from powder. The instructions apply for all filling sizes.

Content

HyClone™ ActiCHO P Powder is a chemically-defined, powdered cell culture medium containing poloxamer 188, without insulin or
L-glutamine. It is hydrolysate-free and animal-derived component-free (ADCF). There are no proteins, peptides or growth factors in ActiCHO P.

Storage and shelf life

Store ActiCHO P Powder dry and protected from light at 2°C to 8°C.

Refer to the product label for the expiry date.

Required materials

- Mixing vessel
- Stirrer

  Note: *A magnetic stirrer bar can be used for small-scale reconstitutions (up to 5 L). An overhead or bottom-mounted impeller is recommended for larger volumes.*
- Calibrated pH meter
- Calibrated osmometer
- Highly purified water, such as WFI (water for injection)

Preparation of liquid medium

Step	Action
1	Fill a clean mixing vessel to 96% of the final volume with high quality purified water, such as WFI (water for injection) at ambient temperature (18°C to 25°C) and start stirring. For example, to prepare a 1 L solution of ActiCHO P take approximately 960 mL of water. ***Note:*** *The stirring should be vigorous enough to quickly draw the powder below the liquid surface, but not too fast to cause formation of air bubbles and excessive foaming.*
2	Add 22.36 g/L ActiCHO P Powder slowly to the vessel, avoiding formation of clumps. Stir for 30 minutes. *Result:* The solution will remain cloudy in this step, but should be clear of any clumps or dry powder residues.
3	Slowly add 6.5 mL/L of a 5 N NaOH solution or 3.25 mL/L of a 10 N NaOH solution and continue stirring for 60 minutes. *Result:* After this step the solution will be clear.
4	Add 1.8 g/L sodium bicarbonate ($NaHCO_3$) into the vessel and continue stirring for 10 minutes.
5	Adjust the pH to between 6.90 and 7.35 by dropwise addition of 5 N or 10 N NaOH. After adjusting, continue stirring for an additional 60 minutes to ensure that all components are completely dissolved. ***Note:*** *The pH will gradually increase with longer mixing times.*
6	Adjust to the final volume with high quality purified water, such as WFI (water for injection) and stir for an additional 10 minutes.
7	Measure and record the final pH and osmolality. *Result:* pH 6.90 to 7.55 Osmolality 285 to 330 mOsmol/kg

Step	Action
8	Sterilize immediately by membrane filtration. Use a low-binding filter membrane type, such as PVDF, PES or cellulose acetate. *Result:* The medium is a clear yellow/brown liquid.
9	Store the medium protected from light at 2°C to 8°C until use.

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