SAFC®
Pharma & Biopharma Raw Material Solutions

EX-CELL® Advanced CHO Fed-batch Medium

Chemically Defined, Animal-Component Free Medium for CHO Cells without L-glutamine, without hypoxanthine, and without thymidine

LIQUID: CATALOG NO. 14366C
POWDER: CATALOG NO. 24366C

Description

EX-CELL® Advanced CHO Fed-Batch Medium is a chemically defined, next generation medium. The formulation was developed using multivariate analysis of 10,000+ data points that included performance, physical, regulatory and safety design specifications. This medium is designed to be used in conjunction with EX-CELL® Advanced CHO Feed 1 for superior platform performance in fed-batch cultures on all industrial CHO cell lineages (CHO-S, DuxB11, DG44, CHO-M, and CHOZN GS).

Intended Use

This product is intended for Further Manufacturing Use in the bio-manufacturing industry. It is not intended or approved for *in-vitro* diagnostic use in the human or veterinary industries.

Product Handling & Storage

Do not use if liquid medium is cloudy or contains precipitates. Powder product should be stored dry at 2–8 °C and protected from light. Liquid product should be stored at 2–8 °C and protected from light for up to one year. Use aseptic technique when handling or supplementing this medium. Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Methods for Use

Preparation Instructions for Powder, Catalog No. 24366C

1. Measure out 80% of final required volume of Milli-Q® or similar purified water intended for cell culture use. Recommended water temperature is 25–40 °C. While stirring, slowly add the powder medium at 22.09 g/L.
2. Continually stir for 15 minutes. Product will remain slightly turbid.
3. Adjust pH to 5.0.
4. Continually stir for 5 minutes. Product will partially clear.
5. Add 1.9 g/L of Sodium Bicarbonate.
6. Continually stir for at least 30 minutes until product is clear.
7. QS to 95% volume.
8. Adjust the pH to 7.2 (range 7.1–7.3).
9. QS to 98% volume.
10. Measure osmolality. Product should be 280–320 mOsm/kg.
11. QS to final volume.
12. Immediately sterile filter with low protein binding filter membrane. (< 0.22 microns)
13. Store product at 2–8 °C in the dark until use.

MilliporeSigma

EX-CELL® Advanced CHO Fed-Batch Medium is formulated without L-glutamine and without hypoxanthine/thymidine. Aseptic supplementation instructions:

1. Add L-glutamine (Catalog No. 59202C), 2–8 mM final concentration, before use in applications not requiring GS selection.
2. Add HT Media Supplement (Catalog No. H0137) before use in applications not requiring DHFR selection.

Note: Shelf life of the product may be affected by the nature of the supplements.

Initiating Cultures

1. Rapidly thaw (<1 minute) a vial of frozen cells in a 37 °C water bath.
2. Transfer the entire contents aseptically into a 125-mL shake flask containing 30 mL prewarmed complete EX-CELL® Advanced CHO Fed-Batch Medium.
3. Incubate at 37 °C in a humidified atmosphere of 5% CO_2 in air on an orbital shaker platform (19 mm diameter orbit) rotating at 120–140 rpm.
4. Maintain cell density between 0.5–1 $\times 10^6$ viable cells/mL for the first two passages following recovery; thereafter, return to your normal maintenance schedule.

Subculturing

1. Verify that the incubator is set to 37 °C, 5% CO_2, and has water for humidity control (~80%).
2. Pre-warm complete EX-CELL® Advanced CHO Fed-Batch Medium to room temperature.
3. Aseptically remove a small volume of cell culture sample from the flask and count by trypan blue exclusion using a hemocytometer or an automated cell counter. Do not proceed if cell viability is less than 90%. Note: If cell viability is below 90%, troubleshoot conditions prior to continuing.
4. Determine the correct volume of cell culture to inoculate a new flask at a starting cell density of 2–3 $\times 10^5$ cells/mL in a total volume of 30 mL fresh EX-CELL® Advanced CHO Fed-Batch Medium per 125-mL shake flask.
5. Aseptically transfer the appropriate amount of cells to the new flask, and add pre-warmed medium up to the desired volume.
6. Incubate flasks in a humidified 37 °C incubator with 5% CO_2 on an orbital shaker at 120–140 rpm.
7. Passage cells by repeating the above steps at least twice weekly, and expand culture volume as necessary. Note: When passing the cells, medium carryover should not exceed 25% of the final volume. If carryover exceeds 25%, centrifugation is recommended.

Cryopreservation

1. Prepare the desired quantity of cells, harvesting in mid-logarithmic phase of growth with viabilities >90%.
2. Prepare a freezing medium consisting of 46.5% cold EX-CELL® Advanced CHO Fed-Batch Medium, 46.5% conditioned medium and 7% dimethyl sulfoxide (DMSO).
3. Harvest cells by centrifugation at 200 g for five minutes. Remove the supernatant.
4. Resuspend cell pellet in the freezing medium at 5 $\times 10^6$ to 1 $\times 10^7$ cells/mL.
5. Rapidly transfer 1–2 mL of this suspension to sterile cryovials.
6. Place the vials in a controlled rate freezing apparatus following standard procedures (1 °C decrease per minute).
7. For long-term storage, transfer the vials to liquid nitrogen (vapor phase).

How to Order

For additional information, please contact your Regional representative or call Customer Service at or visit our website at **SigmaAldrich.com/CHOperformance**

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