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Product Information

EX-CELL® CD CHO Fusion

Serum-Free Medium for CHO Cells
Chemically Defined, Animal-Component Free

without L-glutamine

CATALOG NO. 14365C

Description
EX-CELL CD CHO Fusion is a chemically defined, animal-component free medium developed for the long-term growth of Chinese Hamster Ovary (CHO) cells. The absence of any large macro molecules allows for isolation and purification of secreted proteins from the cells. This medium is supplied without L-glutamine to aid in media stability, to avoid L-glutamine degradation that causes ammonia build-up and to provide an appropriate medium for the culture of CHO cells using glutamine synthetase selection (example: SAFC's CHOZN GS knockout cell line). This medium does not contain hypoxanthine or thymidine to allow for its use with dihydrofolate reductase(DHFR-) gene amplification systems (example: SAFC's CHOZN DHFR knockout cell line).

Formulation
The formula for EX-CELL CD CHO Fusion is proprietary to SAFC. For additional information call our Technical Services department.

Precautions
Use aseptic technique when handling or supplementing this medium. This product is for research or for further manufacturing use. THIS PRODUCT IS NOT INTENDED FOR HUMAN OR THERAPEUTIC USE.

Storage
Store liquid medium at 2 to 8 °C, protected from light. Do not use after the expiration date.

Indications of Deterioration
Medium should be clear and free of particulate and flocculent material. Do not use if liquid medium is cloudy or contains precipitates. Other evidence of deterioration may include color change, pH shift or degradation of physical or performance characteristics.

Preparation Instructions
EX-CELL CD CHO Fusion is formulated with sodium bicarbonate and without L-glutamine. For applications requiring the use of L-glutamine, supplement prior to use with 4 - 8 mM L-glutamine by adding 20 - 40 mL/L of a 200 mM solution (Catalog No. 59202C). SAFC recommends L-glutamine supplementation of the working volume only. Supplements, such as antibiotics, can be added to the sterilized medium using aseptic technique. Storage conditions and shelf life of the product may be affected by the nature of the supplement.

Methods for Use
The following procedure is suggested for cells coming from other formulations that are not EX-CELL CD CHO Fusion.

1. Subculture actively growing cells by planting new cultures at 4 x 10^5 cells/mL in 20 - 30 mL of EX-CELL CD CHO Fusion.
2. Subculture stocks every three to four days at a seeding density of 4 x 10^5 cells/mL.
3. Continue stocks for four to six passages until viabilities stabilize at >90%.
4. Once cells are fully adapted to EX-CELL CD CHO Fusion seeding densities can be adjusted to lower densities for initiating new cultures.

Culture Techniques
Once cultures are fully adapted, the cells should be passed every 3 - 4 days at a seeding density of at least 2-4 x 10^5 cells/mL. An optimal seeding density should be determined by the researcher for each application and cell type. When passing the cells, medium carryover should not exceed 25% of the final volume. If carryover exceeds 25%, centrifugation is recommended. Cells propagated in serum-free or protein-free media are extremely fragile. Standard techniques of centrifugation must be modified to include low-speed centrifugation to prevent damage to cells that have been propagated in serum-free medium.

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Cryopreservation

Freezing:
Cells can be frozen in EX-CELL® CD CHO Fusion without the reintroduction of serum.

1. Choose a culture in logarithmic growth with viabilities above 90%.
2. Prepare a freezing medium consisting of 45% cold EX-CELL CD CHO Fusion medium, 45% spent medium and 10% dimethyl sulfoxide (DMSO).
3. Centrifuge the cells at 200 g for 5 minutes. Remove the supernatant.
4. Resuspend the cells in the freezing medium at 5 x 10^6 to 1 x 10^7 cells/mL.
5. Rapidly transfer 1 - 2 mL of this suspension to sterile cryovials.
6. Place the vials at -20 C for 3 - 4 hours, then transfer to -70 °C for 16 - 24 hours.
7. For long-term storage, transfer the vials to liquid nitrogen vapor.

Thawing:
1. Rapidly thaw a vial of frozen cells in a 37 °C water bath.
2. Transfer the cells aseptically to a centrifuge tube containing 10 mL of chilled EX-CELL CD CHO Fusion medium.
3. Using low-speed centrifugation, pellet the cell suspension at 200 g for 5 minutes and carefully decant the supernatant without disturbing the cell pellet.
4. Resuspend the cells in 5 mL of EX-CELL CD CHO Fusion medium.
5. Count the cells for viability and transfer to a sterile tissue culture flask at a seeding density of 4 x 10^5 cells/mL.
6. When cell densities reach 1-2 x 10^6 cells/mL, passage the cells using standard cell culture techniques.

Characteristics

- **Appearance** – Clear solution
- **Osmolality (as supplied)** – 292 - 322 mOsm/kg H_2O
- **pH (as supplied)** – 7.1 - 7.7
- **Sterility** – No microbial growth detected

Product Profile

SAFC EX-CELL CD CHO Fusion was compared to five chemically defined competitor formulations designed for CHO cells (Table 1). Three proprietary rIgG-producing CHO cell lines were used for the purposes of these comparisons.

NOTE: All cell lines were adapted to each formulation by passing six times before evaluating growth and productivity. Cell line 2 could not be adapted to GIBCO CD CHO.

Table 1: CHO Formulations Used for Comparison Assays

Medium	Manufacturer	Cat No.
EX-CELL CD CHO Fusion	SAFC	14365C/24365C
CD CHO Medium	GIBCO (Life Technologies)	10743
CD OptiCHO™ Medium	GIBCO (Life Technologies)	12681
PowerCHO®-2	BioWhittaker (Lonza)	12-771Q
CDM4CHO™	HyClone (Thermo Scientific)	SH30558
IS CHO-CD4™	Irvine Scientific	9110

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