



# EX-CELL® CD CHO Fusion

## Chemically defined cell culture medium

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: Merck'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: Merck's CHOZN DHFR knockout cell line).

### Formulation

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

### Precautions

Use aseptic technique when handling or supplementing this medium.

This product is intended for research or further manufacturing but not for human or therapeutic use.

### Indications of Deterioration

Dry powder medium should be free flowing. Do not use if medium is caked. Liquid 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.

### Storage

Store dry powder medium at 2–8°C protected from light.

Store liquid medium at 2–8°C protected from light.

Do not use after expiration date.

### Preparation Instructions

Dry powder medium is vacuum dried where appropriate during the particle reduction process and packaged in a humidity-controlled environment. This treatment ensures maximum dehydration and product stability. The end product is extremely hygroscopic and must be protected from atmospheric moisture.

We recommend that the entire contents of each package be used immediately after opening. Preparing concentrated solutions is not recommended because of the low solubility coefficients of some amino acids and the tendency of some salts to form insoluble complexes.

EX-CELL® CD CHO Fusion dry powder medium is formulated without L-glutamine and without sodium bicarbonate.

## Media Hydration

1. Measure 80–90% of final required volume of cell culture grade water (Catalog No. 59900C) into an appropriate sized mixing vessel. Water temperature should be 20 to 30 C.
2. Slowly add 20.09 g/L of EX-CELL® CD CHO Fusion dry powder medium. Rinse the package with a small amount of cell culture grade water to remove traces of powder and add to the solution.
3. Mix for at least 20 minutes, or until completely dissolved. Do not heat the medium.
4. Add 1.25 g/L of sodium bicarbonate (Catalog No. 90421C). Mix until completely dissolved.
5. While mixing, adjust the pH to 7.2 – 7.4 using NaOH 1N or HCl 1N.
6. Add cell culture grade water to the solution to bring it to final volume and continue mixing for at least 60 minutes. To avoid fluctuations in pH, keep the vessel closed until the medium is filtered.
7. Sterile filter using a low protein-binding membrane filter with a pore size of 0.22 µm. For larger volumes, a lowprotein binding 0.45 µm pre-filter is recommended. To minimize CO<sub>2</sub> loss, a peristaltic pump or an inert gas, such as nitrogen, can be used to provide positive pressure at 2 – 15 psi. Do not use CO<sub>2</sub> gas.

**Note:** 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). Merck 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  $4 \times 10^5$  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  $4 \times 10^5$  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  $2\text{--}4 \times 10^5$  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.

## Cryopreservation

### Cell freezing operation procedure:

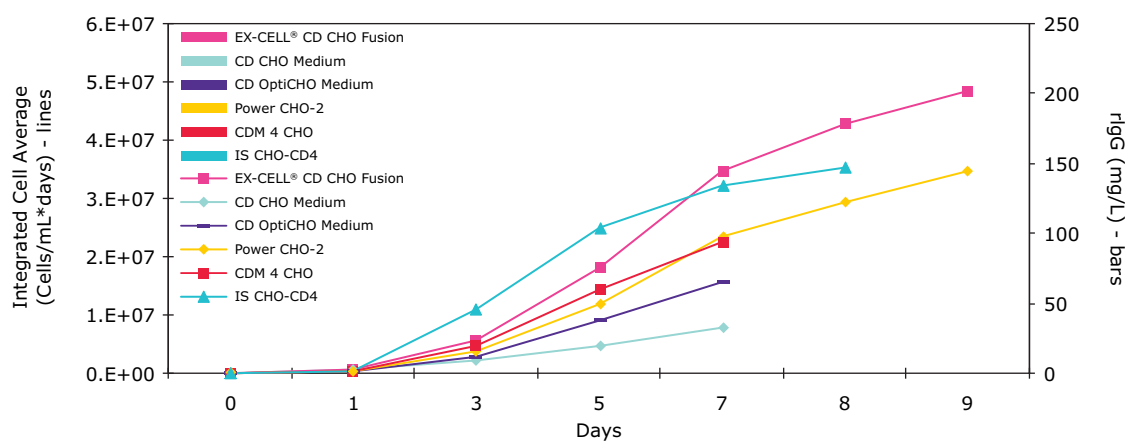
Cells can be frozen in EX-CELL® CD CHO Fusion without the reintroduction of serum.

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

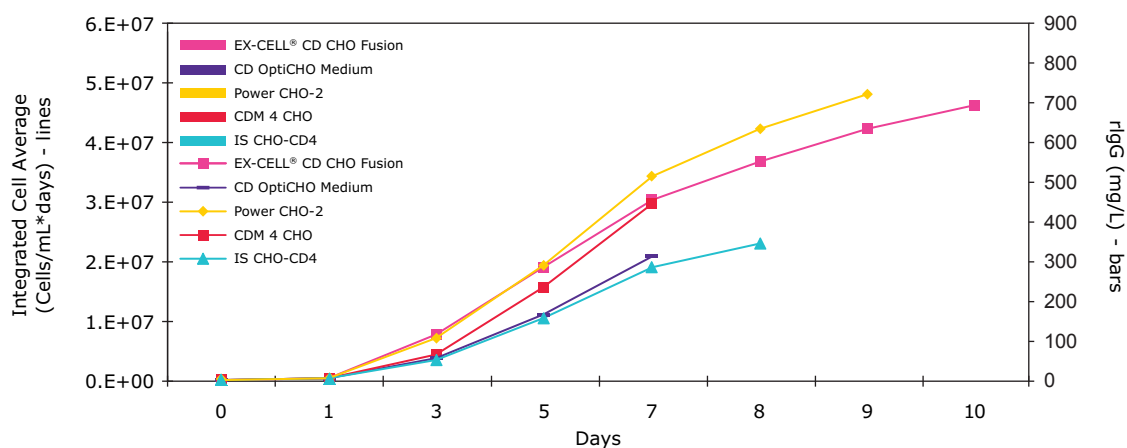
### Cell thawing and recovery procedure:

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

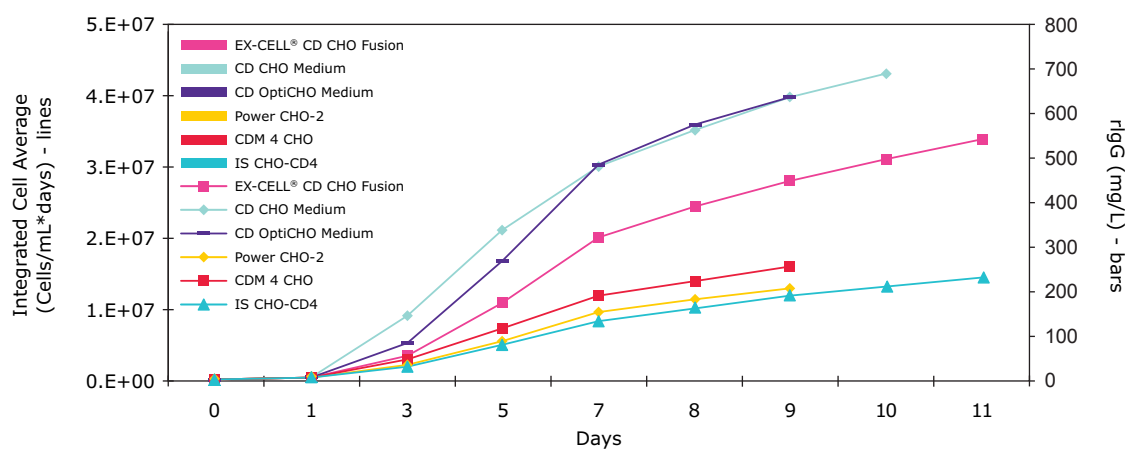
## Cell Line 1



## Cell Line 2



## Cell Line 3



## Product Profile

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.

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

**Table 1: CHO Formulations Used for Comparison Assays**

**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.

## Ordering Information

Cat. No.	Product Name
24365C	EX-CELL® CD CHO Fusion (Dry Powder)
14365C	EX-CELL® CD CHO Fusion (Liquid)
S5761	Sodium Bicarbonate
59202C	L-Glutamine Solution 200 mM

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

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Germany: 069 86798021  
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