SAFC Biosciences™
Accelerate Success™

Product Information

EX-CELL™ CD CHO Fusion Serum-Free Medium for CHO Cells Chemically Defined, Animal-Component Free

without L-glutamine, without sodium bicarbonate

CATALOG NO. 24365C

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 macromolecules 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 the Glutamine Synthetase, or GS, System™. This medium does not contain hypoxanthine or thymidine to allow for its use with dihydrofolate reductase ($DHFR^-$) gene amplification systems.

Formulation

The formula for EX-CELL™ CD CHO Fusion is proprietary to SAFC Biosciences™. 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 dry powder medium at 2 to 8 C. Store hydrated medium at 2 to 8 C, protected from light. Do not use after the expiration date.

Indications of Deterioration

Medium should be free flowing. Do not use if medium is caked. Hydrated 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

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 is formulated without L-glutamine and without sodium bicarbonate.

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.

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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 low-protein binding 0.45 µm pre-filter is recommended. To minimize CO_2 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_2 gas.

   NOTE: For applications requiring the use of L-glutamine, supplement with 4 - 8 mM L-glutamine by adding 20 - 40 mL/L of a 200 mM solution (Catalog No. 59202C) prior to use. SAFC Biosciences recommends L-glutamine supplementation of the working volume only. Other supplements, such as antibiotics, can be added to the sterilized medium using aseptic technique. Storage conditions and shelf life of the supplemented product may be affected by the nature of the supplements.

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.

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 and 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 106 cells/mL, passage the cells using standard cell culture techniques.

Characteristics

Appearance
Free flowing powder

Osmolality (as supplied)
Refer to Certificate of Analysis

pH (as supplied)
Refer to Certificate of Analysis

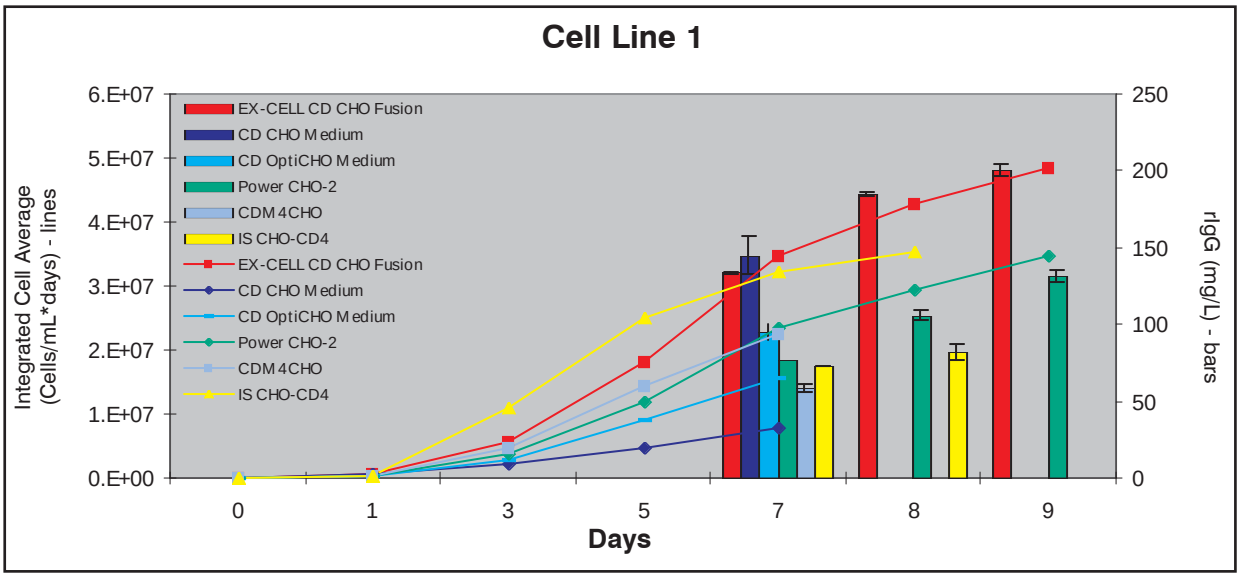
Product Profile

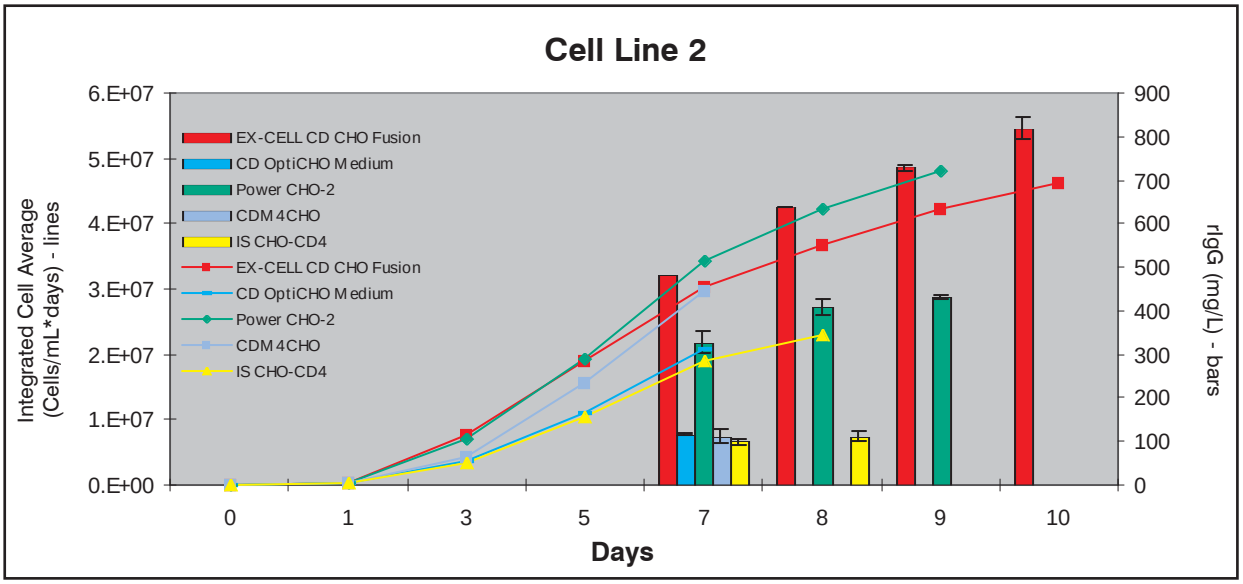
SAFC Biosciences EX-CELL™ CD CHO FUSION was compared to five chemically defined competitor formulations designed for CHO cells (Table 1). Three proprietary rIgG-producing CHO cell lines were used for the purposes of these comparisons.

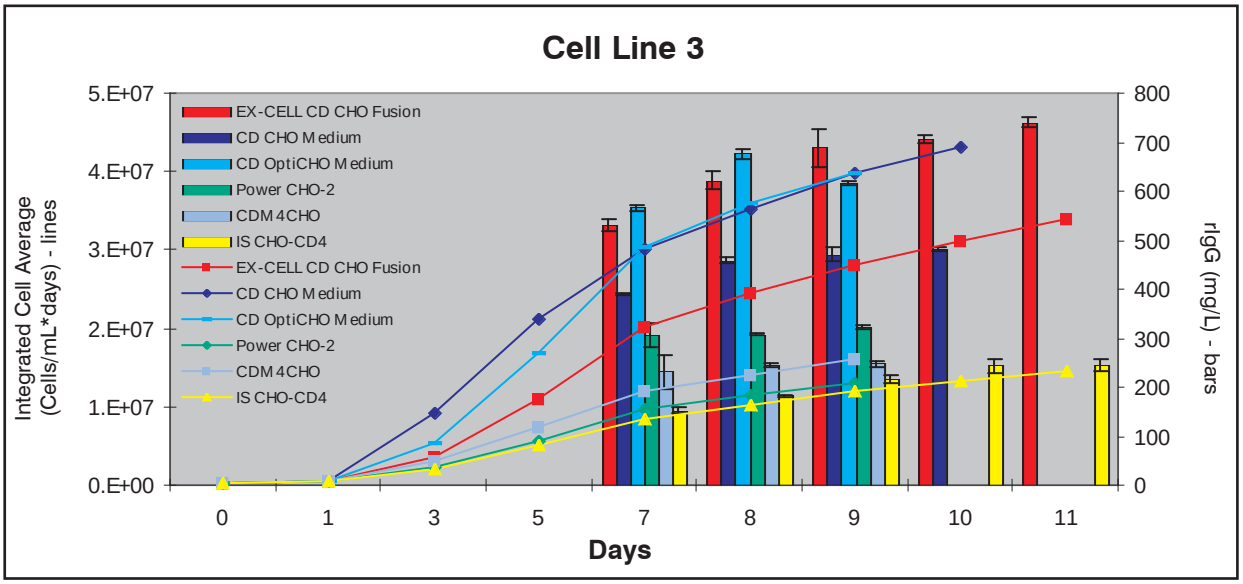
NOTE: All cell lines were adapted to each formulation by passing six times before evaluating growth and productivity. Cell line 2 could not be adapted to GIBCO CD CHO.

Table 1: CHO formulations used for comparison assays.

MEDIUM	MANUFACTURER	CATALOG NO.
EX-CELL™ CD CHO FUSION	SAFC Biosciences	14365C/24365C
CD CHO Medium	GIBCO	10743
CD OptiCHO™ Medium	GIBCO	12681
PowerCHO®-2	BioWhittaker (Lonza)	12-771Q
CDM4CHO™	HyClone (Thermo Scientific)	SH30558
IS CHO-CD4™	Irvine Scientific	91100


Cell Line 1
Integrated Cell Average (Cells/mL*days) - lines
rIgG (mg/L) - bars
Days
EX-CELL CD CHO Fusion
CD CHO Medium
CD OptiCHO Medium
Power CHO-2
CDM4CHO
IS CHO-CD4
6.E+07
5.E+07
4.E+07
3.E+07
2.E+07
1.E+07
0.E+00
250
200
150
100
50
0
0
1
3
5
7
8
9


Cell Line 2
Integrated Cell Average (Cells/mL*days) - lines
rIgG (mg/L) - bars
Days
EX-CELL CD CHO Fusion
CD OptiCHO Medium
Power CHO-2
CDM4CHO
IS CHO-CD4
6.E+07
5.E+07
4.E+07
3.E+07
2.E+07
1.E+07
0.E+00
900
800
700
600
500
400
300
200
100
0
0
1
3
5
7
8
9
10


Cell Line 3
Integrated Cell Average (Cells/mL*days) - lines
rIgG (mg/L) - bars
Days
EX-CELL CD CHO Fusion
CD CHO Medium
CD OptiCHO Medium
Power CHO-2
CDM4CHO
IS CHO-CD4
5.E+07
4.E+07
3.E+07
2.E+07
1.E+07
0.E+00
800
700
600
500
400
300
200
100
0
0
1
3
5
7
8
9
10
11

Warranty, Limitation of Remedies

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Additional Terms and Conditions are contained in the product Catalog, a copy of which is available upon request.

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