SAFC Biosciences™
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Product Information

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

without L-glutamine, without hypoxanthine, without thymidine, without sodium bicarbonate

CATALOG NO. 24360C

Description

EX-CELL™ CD CHO is a chemically defined, animal-component free, serum-free dry powder medium developed for the long-term growth of Chinese Hamster Ovary (CHO) cells and expression of antibodies or protein products in suspension culture. CHO suspension cultures can be subcultured directly into EX-CELL™ CD CHO from serum-supplemented or serum-free medium with little or no adaptation. EX-CELL™ CD CHO (Catalog No. 24360C) is formulated without hypoxanthine, thymidine and L-glutamine, making it an appropriate medium for selection systems such as DHFR$^-$ and Glutamine Synthetase (GS System™). For applications that do not require the selective pressure of a hypoxanthine/thymidine (HT)-deficient medium, we recommend the use of EX-CELL™ CD CHO with hypoxanthine and thymidine (Catalog No. 24361C).

Formulation

The formula for EX-CELL™ CD CHO is proprietary to SAFC Biosciences. For additional information please 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 particulates and flocculent material. Do not use if liquid medium is cloudy or contains precipitate. 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 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 19.24 g/L of EX-CELL™ CD CHO dry powder medium, allowing mixing time between additions. 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 30 minutes (product will be hazy). Do not heat the medium.

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4. While mixing the solution, adjust the pH to 8.5 - 9.0 using NaOH 1N (Catalog No. 59223C). Mix for 15 minutes and then adjust the pH to 6.8 using HCl 1N before adding sodium bicarbonate.
5. Add 2.1 g/L of sodium bicarbonate (Catalog No. 90421C) or 28.5 mL/L of sodium bicarbonate solution 7.5% (Catalog No. 59221C). Mix until completely dissolved.
6. While mixing, adjust the pH to 6.9 - 7.1 using NaOH 1N or HCl 1N. The pH of this medium usually rises 0.1 - 0.2 units during filtration. For most applications, the optimal pH of the filtered medium is 7.0 - 7.4.
7. 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.
8. To sterilize the medium, 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 8 mM L-glutamine by adding 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.
9. Dispense medium into sterile containers using aseptic technique. Store liquid medium protected from light at 2 to 8 C.

Methods for Use

Adaptation

CHO cells that have been grown in attachment or suspension cultures in a conventional serum-supplemented medium can be readily grown in EX-CELL™ CD CHO with little or no adaptation. Adaptation to EX-CELL™ CD CHO requires healthy, viable cultures in mid-logarithmic growth phase. During adaptation, growth rates can be somewhat slower than normal expected results.

Adaptation from attachment cultures

1. Subculture the cells from serum-supplemented medium to EX-CELL™ CD CHO supplemented with 8 mM L-glutamine using standard trypsinization techniques when cultures reach 100% confluence.
2. Inactivate the trypsin with medium containing 5% gamma irradiated Fetal Bovine Serum (FBS) (Catalog No. 12107C). Using low-speed centrifugation, pellet the cell suspension at 200 *g* for 5 minutes and carefully decant the supernatant without disturbing the cell pellet.
3. Resuspend the cells in EX-CELL™ CD CHO medium supplemented with 8 mM L-glutamine at a density of 5 x 10^5 cells/mL in 25 cm^2 or 75 cm^2 flasks.
4. Incubate the cells at 37 C in a humidified incubator with 8 - 10% CO_2.
5. Continue to subculture the cells in EX-CELL™ CD CHO every 3 - 4 days for 3 - 4 passages using a seeding density of 5 x 10^5 cells/mL in 25 cm^2 or 75 cm^2 flasks. SAFC Biosciences recommends centrifugation of the cell suspension prior to passaging.
6. After 3 - 4 passages, centrifuge and resuspend the cells in EX-CELL™ CD CHO medium supplemented with 8 mM L-glutamine at a density of 3-5 x 10^5 cells/mL in 125 mL shaker flasks.
7. Continue to subculture cells in EX-CELL™ CD CHO every 3 - 4 days as described in step No. 6.
8. Allow the cells to adapt to EX-CELL™ CD CHO for an additional 4 - 6 passages. Cells are considered fully adapted to EX-CELL™ CD CHO when growth rates return to normal and viabilities are above 95%.
9. Cells can now be scaled up as necessary using standard procedures and densities.

Adaptation from suspension cultures

1. Resuspend the cells in EX-CELL™ CD CHO medium supplemented with 8 mM L-glutamine at a density of 5 x 10^5 cells/mL in 125 mL shaker flasks.
2. Incubate the cells at 37 C in a humidified incubator with 8 - 10% CO_2.
3. Continue to subculture the cells in EX-CELL™ CD CHO every 3 - 4 days for 3 - 4 passages using a seeding density of 5 x 10^5 cells/mL in 125 mL flasks. SAFC Biosciences recommends centrifugation of the cell suspension prior to passaging.
4. After 3 - 4 passages, centrifuge and resuspend the cells in EX-CELL™ CD CHO medium supplemented with 8 mM L-glutamine at a density of 3-5 x 10^5 cells/mL in 125 mL shaker flasks.

5. Continue to subculture cells in EX-CELL™ CD CHO every 3 - 4 days as described in step No. 4.
6. Allow the cells to adapt to EX-CELL™ CD CHO for an additional 4 - 6 passages. Cells are considered fully adapted to EX-CELL™ CD CHO when growth rates return to normal and viabilities are above 95%.
7. Cells can now be scaled up as necessary using standard procedures and densities.

Culture Techniques

CHO cells are normally grown at 37 ± 1 C and 5 - 10% CO_2. Allow the medium to warm to room temperature prior to use (protect from light). Once fully adapted, the cells should be subcultured at a seeding density of at least 3 x 10^5 cells/mL in shaker flasks. Seed 30 mL cell cultures in 125 mL shaker flasks and 60 mL cultures in 250 mL shaker flasks. Shaker speed should be approximately 125 rpm ± 5 rpm. Optimal seeding densities and L-glutamine concentrations should be determined by the researcher for each application and cell type.

When passing the cells, medium carry over should not exceed 25% of the final volume. If carry over exceeds 25%, centrifugation is recommended. For best results, centrifugation is recommended regardless of medium carryover. Cells propagated in serum-free media are extremely fragile. For successful results, care must be taken when subculturing cells. 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.

During adaptation, normal trypsin concentrations may be used, but incubations should be carried out at 4 C, and exposure time should be minimal. SAFC Biosciences recommends the use of a soybean trypsin inhibitor (0.1%), or sedimentation by centrifugation to remove the trypsin. Soybean trypsin inhibitor should be used with caution, as it is toxic to some cell types. Cells may also be dislodged with NO-ZYME™ (Catalog No. 59226C), a non-enzymatic dissociating agent.

Cryopreservation

Freezing:

Cells can be frozen in EX-CELL™ CD CHO without the reintroduction of serum. However, it is necessary to handle the cells gently and freeze the cells under carefully controlled conditions.

1. Choose cultures in logarithmic growth with viabilities above 90%.
2. Prepare a freezing medium consisting of 45% cold EX-CELL™ CD CHO medium, 45% conditioned medium and 10% dimethyl sulfoxide (DMSO). Alternatively, cells can be frozen in 90% fresh medium and 10% DMSO.
3. Centrifuge the cells at 200 *g* for 5 minutes. Remove the supernatant.
4. Resuspend the cells in the freezing medium at 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 5 - 10 mL of cold EX-CELL™ CD CHO 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 medium.
5. Count the cells for viability and transfer to a sterile shaker flask at a seeding density of 4 x 10^5 cells/mL.

Characteristics

Appearance

Off-white to pink free-flowing powder

Endotoxin

Refer to Certificate of Analysis

Osmolality (as supplied)

Refer to Certificate of Analysis

pH (as supplied)

Refer to Certificate of Analysis

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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