SAFC Biosciences™
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Product Information

EX-CELL™ 610-HSF Serum-Free Medium for Hybridoma Cells

with L-glutamine, without sodium bicarbonate

CATALOG NO. 24610C

Description

EX-CELL™ 610-HSF is a low-protein (11 mg/L) serum-free dry powder medium. Originally developed to support the growth of hybrid cells in culture, it has been shown to support a wide range of cells including lymphoid and epithelial cells and B cell hybridomas of murine, rat and human origin. EX-CELL™ 610-HSF has been used in stationary culture systems and in large-scale bioreactors. In both types of culture, the production of cellular products, particularly monoclonal antibodies, has been shown to equal or exceed levels seen when the same cells are cultured in the presence of Fetal Bovine Serum (FBS).

Formulation

The formulation for EX-CELL™ 610-HSF is proprietary to SAFC Biosciences. For additional information please call our Technical Services department.

Precautions

Use aseptic technique when handling or supplementing this medium after filtration. 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™ 610-HSF is formulated with 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 size mixing vessel. Water temperature should be 20 to 30 C.
2. Slowly add 15.08 g/L of EX-CELL™ 610-HSF dry powder medium. Stir until completely dissolved. Rinse the package with a small amount of cell culture grade water to remove traces of powder and add to the solution.
3. Mix until completely dissolved. Do not heat the medium.
4. Add 1.6 g/L of sodium bicarbonate (Catalog No. 90421C) or 4.7 mL/L of sodium bicarbonate solution 7.5% (Catalog No. 59221C). Mix until dissolved.
5. While mixing the solution, adjust the pH to 6.9 - 7.1 using NaOH 1N (Catalog No. 59223C) or HCl 1N. The pH of this medium usually rises 0.1 - 0.2 units during the filtration. For most applications, the optimal pH of the filtered medium is 7.0 - 7.4.
6. Add cell culture grade water to the solution to bring it to final volume. Continue mixing for at least 60 minutes. To avoid fluctuation in pH, keep the vessel closed until the medium is filtered.

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7. 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: Other supplements, such as antibiotics or L-glutamine, 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.

8. Dispense medium into sterile containers using aseptic technique. Store liquid medium protected from light at 2 to 8 C.

Methods for Use

Adaptation

1. Culture the cells to a density of 3-5 x 10^5 cells/mL in EX-CELL™ 610-HSF medium containing 5% gamma irradiated Fetal Bovine Serum (FBS) (Catalog No. 12107C). Maintain the cells through 2 passages at this concentration of serum.
2. Subculture into EX-CELL™ 610-HSF containing 2.5% serum and maintain through 2 passages with the same concentration of serum.
3. Subculture into EX-CELL™ 610-HSF containing 1% serum and maintain through 2 passages in the same serum concentration.
4. If no major growth changes in the cells are observed, pass the cells into EX-CELL™ 610-HSF serum-free medium.
5. Once cells are growing in EX-CELL™ 610-HSF without serum, they can be subcultured every 3 - 4 days as necessary.

Culture Techniques

Once cultures are fully adapted, the cells should be passed every 3 - 4 days at a seeding density of at least 2 x 10^5 cells/mL. An optimal seeding density should be determined by the researcher for each application and cell type. EX-CELL™ 610-HSF does not contain cholesterol. If using a cholesterol-dependent cell line, supplement with 10 - 20 µm cholesterol (3.8 - 7.7 mg/L).

When passing the cells, carryover should not exceed 25% of the final volume. If carryover exceeds 25%, centrifugation is recommended. Cells propagated in serum-free medium are extremely fragile. Standard techniques for 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™ 610-HSF without the reintroduction of serum.

1. Choose cultures in logarithmic growth with viabilities above 90%.
2. Prepare a freezing medium consisting of 45% cold EX-CELL™ 610-HSF 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 cold EX-CELL™ 610-HSF.
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™ 610-HSF.
5. Count the cells for viability and transfer to a sterile shaker flask at a seeding density of 2-4 x 10^5 cells/mL.
6. When the culture has reached a density of 1 x 10^6 cells/mL, passage the cells using standard cell culture techniques.

Characteristics

Appearance
Off-white free-flowing powder

Bioburden
≤ 500 CFU/100 mL

Endotoxin
≤ 10.0 EU/mL

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.

Issued September 2006 P24610
1103 0805 0905 0406

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