

# Product Information

## EX-CELL™ 620-HSF Serum-Free Medium for Hybridoma Cells

without L-glutamine, without sodium bicarbonate

CATALOG NO. 24621C

### Description

EX-CELL™ 620-HSF is a low-protein (approximately 11 mg/L) serum-free medium which has been specifically developed for the long-term growth of hybridoma and related cells capable of expressing monoclonal antibodies and other protein products. The following hybridoma, lymphoma and myeloma cell lines have demonstrated sustained long-term, serum-free growth (> 15 passages) in EX-CELL™ 620-HSF: Sp2/0 Ag14, L243, VD-10, Hut 78, K562. Additionally, transformed Chinese Hamster Ovary (CHO) cells have demonstrated excellent growth and productivity when grown in this medium.

### Formulation

The formulation for EX-CELL™ 620-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. 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 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™ 620-HSF 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 size mixing vessel. Water temperature should be 20 to 30 C.
2. Slowly add 17.16 g/L of EX-CELL™ 620-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  $\mu\text{m}$ . For larger volumes, a low-protein binding 0.45  $\mu\text{m}$  pre-filter is recommended. To minimize  $\text{CO}_2$  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  $\text{CO}_2$  gas.

**NOTE:** For applications requiring the use of L-glutamine, supplement with 4 mM L-glutamine by adding 20 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.

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

## Methods for Use

### Adaptation

Mammalian cells can be adapted to serum-free conditions by direct adaptation from a serum-containing medium or by gradual weaning. Both procedures require healthy, viable cultures in mid-logarithmic growth phase. During the adaptation phase growth rates will usually be somewhat slower than growth in serum-containing medium.

#### *Gradual Weaning to Serum-Free Media (Recommended):*

1. Passage cells from the serum-containing medium directly into EX-CELL™ 620-HSF + 5% gamma irradiated Fetal Bovine Serum (FBS) (Catalog No. 12107C) at the recommended seeding density.
2. After 2 passages at the 5% FBS concentration subculture the cells into EX-CELL™ 620-HSF + 1% FBS using the recommended seeding density.
3. Allow the cells to adapt to EX-CELL™ 620-HSF + 1% FBS for an additional 2 - 3 passages, then subculture the cells into EX-CELL™ 620-HSF + 0.5% FBS.
4. The cells can then be subcultured into EX-CELL™ 620-HSF.

#### *Direct Adaptation to Serum-Free Media:*

1. Passage the cells into pre-warmed (37 C) EX-CELL™ 620-HSF at 1.5-2X the recommended seeding density.
2. Refeed the culture after 48 hours with a 100% exchange of fresh EX-CELL™ 620-HSF medium.
3. Allow the cultures to become 90% - 100% confluent before subculturing or during mid-logarithmic growth phase.
4. Subculture into fresh EX-CELL™ 620-HSF as in Step 1 using normal seed densities.

## Culture Techniques

Once cultures are fully adapted, the cells should be passed every 3 - 4 days at a seeding density of at least  $2 \times 10^5$  cells/mL. An optimal seeding density should be determined by the researcher for each application and cell type.

When passing the cells, 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™ 620-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™ 620-HSF media, 45% spent media 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  $5 \times 10^6$  to  $1 \times 10^7$  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™ 620-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™ 620-HSF.
5. Count the cells for viability and transfer to a sterile shaker flask at a seeding density of  $2-4 \times 10^5$  cells/mL.
6. When the culture has reached a density of  $1 \times 10^6$  cells/mL, passage the cells using standard cell culture techniques.

## Characteristics

### Appearance

Off-white free-flowing powder

### Bioburden

$\leq 500$  CFU/100 mL

### Endotoxin

$\leq 10.0$  EU/mL

### Osmolality (as supplied)

Refer to Certificate of Analysis

### pH (as supplied)

Refer to Certificate of Analysis

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