SAFC Biosciences™
Accelerate Success™

Product Information

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

without L-glutamine

CATALOG NO. 14621C

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 liquid medium at 2 to 8 C, protected from light. Do not use after the expiration date.

Indications of Deterioration

Medium should be clear and free of particulates and flocculent material. Do not use if 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

EX-CELL™ 620-HSF is formulated without L-glutamine. 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. Other supplements, such as antibiotics, can be added to the medium using aseptic technique. Storage conditions and shelf life of the product may be affected by the nature of the supplements.

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.

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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 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 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 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™ 620-HSF 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™ 620-HSF medium.
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
Clear orange-red solution

Endotoxin
≤ 10.0 EU/mL

Osmolality (as supplied)
300 - 340 mOsm/Kg H_2O

pH (as supplied)
7.0 - 7.4

Sterility
No microbial growth detected

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