SAFC Biosciences™
Accelerate Success™

Product Information

EX-CELL™ Sp2/0 Serum-Free Medium for Sp2/0 Cells, Chemically Defined

without L-glutamine

CATALOG NO. 14660C

Description

EX-CELL™ Sp2/0 is an animal-component free, protein-free, chemically defined, serum-free liquid medium developed for the long-term growth of Sp2/0-related cells in suspension culture. The Sp2/0 cells are capable of growth in suspension culture. Sp2/0 hybridoma suspension cultures can be subcultured directly into EX-CELL™ Sp2/0 from serum-supplemented or serum-free media with little or no adaptation. Suspension cultures in EX-CELL™ Sp2/0 have been carried for more than 25 passages with no loss of growth or viability.

Formulation

The formula for EX-CELL™ Sp2/0 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 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

EX-CELL™ Sp2/0 is formulated without L-glutamine. Prior to use, this medium should be supplemented with 8 mM L-glutamine by adding 40 mL/L of a 200 mM solution (Catalog No. 59202C). SAFC Biosciences recommends L-glutamine supplementation of the working volume only. Supplements, such as antibiotics, can be added to the sterilized medium using aseptic technique. Storage conditions and shelf life of the product may be affected by the nature of the supplement.

Methods for Use

Adaptation

Sp2/0 hybridoma cells that have been grown in suspension cultures in a conventional serum-supplemented medium can be readily grown in EX-CELL™ Sp2/0 with little or no adaptation. Sp2/0 cells in suspension culture must be healthy, viable cultures in mid-logarithmic growth phase prior to adaptation.

1. Subculture the cells from serum-supplemented medium to EX-CELL™ Sp2/0 supplemented with 8 mM L-glutamine at a minimum seeding density of 3 x 10^5 cells/mL in shaker flasks.
2. Incubate the flasks at 37 C in a humidified incubator with 10% CO_2. Maintain the orbital shaker speed at approximately 165 rpm.
3. Continue to subculture cells in EX-CELL™ Sp2/0 every 3 - 4 days, using the above seeding density.
4. Allow the cells to adapt to EX-CELL™ Sp2/0 for an additional 3 - 6 passages. Cells are considered fully adapted to EX-CELL™ Sp2/0 when growth rates return to normal and viabilities are above 95%.

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Culture Techniques

Sp2/0 cells are normally grown at 37 ± 1 C and 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 165 rpm.

When passing the cells, medium carry over should not exceed 30% of the final volume. If carry over exceeds 30%, centrifugation is recommended. 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.

Cryopreservation

Freezing:

Sp2/0 cells can be frozen in EX-CELL™ Sp2/0 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 90% cold EX-CELL™ Sp2/0 medium and 10% dimethyl sulfoxide (DMSO).
3. Centrifuge the cells at 200 *g* for 5 minutes. Remove the supernatant and prepare the freezing medium.
4. Gently 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 without agitation.
2. Transfer the cells aseptically to a centrifuge tube containing 5 mL of cold EX-CELL™ Sp2/0 medium.
3. Count the cells for viability and transfer to a sterile shaker flask at a seeding density of 3 x 10^5 cells/mL.
4. Pass the cells using standard cell culture techniques.

Characteristics

Appearance
Clear yellow solution

Endotoxin
Refer to Certificate of Analysis

Osmolality (as supplied)
305 - 335 mOsm/kg H_20

pH (as supplied)
6.9 - 7.3

Sterility
No microbial growth detected

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