SAFC Biosciences™
Accelerate Success™

Product Information

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

without L-glutamine, without sodium bicarbonate

CATALOG NO. 24660C

Description

EX-CELL™ Sp2/0 is an animal-component free, protein-free, chemically defined, serum-free dry powder 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 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™ Sp2/0 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 17.27 g/L of EX-CELL™ Sp2/0 dry powder medium. Mix 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.

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4. Add 1.05 g/L of sodium bicarbonate (Catalog No. 90421C) or 14 mL of sodium bicarbonate solution 7.5% (Catalog No. 59221C). Mix until fully dissolved.
5. While mixing the solution, adjust the pH to 6.6 - 7.0 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 6.9 - 7.3.
6. Check osmolality of EX-CELL™ Sp2/0 medium. The osmolality range should be 305 - 335 mOsm/kg H_20 or can be adjusted with sodium chloride.
7. Add cell culture grade water to the solution to bring it to final volume. To avoid fluctuation 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

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%.

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. 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

Bioburden
Refer to Certificate of Analysis

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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