SAFC Biosciences™
Accelerate Success™

Product Information

EX-CELL™ NS0 Serum-Free Medium for NS0 Cells, Chemically Defined

without L-glutamine, without sodium bicarbonate

CATALOG NO. 24651C

Description

EX-CELL™ NS0 is an animal-component free, protein-free, chemically defined, serum-free dry powder medium developed for the long-term growth of NS0-related cells in suspension culture. The NS0 cells are clonal derivatives of the parent NS1 cell line and are capable of growth in suspension culture. NS0 hybridoma suspension cultures can be subcultured directly into EX-CELL™ NS0 from serum-supplemented or serum-free media. Suspension cultures in EX-CELL™ NS0 have been carried for more than 50 passages with no loss of growth or viability.

Formulation

The formula for EX-CELL™ NS0 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 protected from light at 2 to 8 C. 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™ NS0, Catalog No. 24651C, 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 sized mixing vessel. Water temperature should be 20 to 30 C.
2. Slowly add 18.51 g/L of EX-CELL™ NS0 dry powder media, allowing mixing time between additions. Rinse the package with a small amount of cell culture grade water to remove traces of powder and add to the solution.
3. Mix for at least 30 minutes (product will be hazy). Do not heat the medium.
4. While mixing the solution, adjust the pH to 8.5 - 9.0 using NaOH 1N (Catalog No. 59223C). Mix for 15 minutes and then adjust the pH to 6.8 using HCl 1N before adding sodium bicarbonate.
5. Add 2.1 g/L of sodium bicarbonate (Catalog No. 90421C) or 28.5 mL/L of sodium bicarbonate solution 7.5% (Catalog No. 59221C). Mix until completely dissolved.

United States

SAFC Biosciences, Inc.
13804 W. 107th Street
Lenexa, Kansas 66215
USA
Phone +1 913-469-5580
Toll free-USA 1 800-255-6032
Fax +1 913-469-5584
E-mail info-na@sial.com

Europe

SAFC Biosciences Ltd.
Smeaton Road, West Portway
Andover, Hampshire SP10 3LF
UNITED KINGDOM
Phone +44 (0)1264-333311
Fax +44 (0)1264-332412
E-mail info-eu@sial.com

Asia Pacific

SAFC Biosciences Pty. Ltd.
18-20 Export Drive
Brooklyn, Victoria 3025
AUSTRALIA
Phone +61 (0)3-9362-4500
Toll free-AUS 1 800-200-404
Fax +61 (0)3-9315-1656
E-mail info-ap@sial.com

www.safcbiosciences.com

6. While mixing, adjust the pH to 6.9 - 7.1 using NaOH 1N or HCl 1N. The pH of this medium usually rises 0.1 - 0.2 units during filtration. For most applications, the optimal pH of the filtered medium is 7.0 - 7.4.
7. Add cell culture grade water to the solution to bring it to final volume and continue mixing for at least 60 minutes. To avoid fluctuations 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

NS0 hybridoma cells that have been grown in a conventional serum-supplemented medium can be adapted to EX-CELL™ NS0 directly with no weaning. Adaptation to EX-CELL™ NS0 requires healthy, viable cultures in mid-logarithmic growth phase.

1. Subculture the cells from serum-supplemented medium into EX-CELL™ NS0 at a minimum seeding density of |2 x 10^5 cells/mL in shaker flasks.
2. Incubate the flasks at 37 C in a humidified incubator with 5% CO_2. Maintain the orbital shaker speed between 105 - 115 rpm.
3. Continue to subculture cells in EX-CELL™ NS0 every 3 - 4 days or when the cell density reaches 2 x 10^6 cells/mL.
4. Allow the cells to adapt to EX-CELL™ NS0 for an additional 3 - 6 passages. Cells are considered fully adapted to EX-CELL™ NS0 when growth rates return to normal and viabilities are above 95%.

Culture Techniques

NS0 cells are normally grown at 37 ± 1 C and 5% 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 2 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 between 105 - 115 rpm.

When passing the cells, carry over should not exceed 25% of the final volume. If carry over exceeds 25%, 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:

NS0 cells may be frozen in EX-CELL™ NS0 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 phase with viabilities above 90%.
2. Prepare a freezing medium consisting of 45% cold EX-CELL™ NS0 medium, 45% spent medium and 10% dimethyl sulfoxide (DMSO). Alternatively, cells can be frozen in 90% fresh medium and 10% DMSO. Recovery may require 1 - 2 additional passes.
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™ NS0 media.
3. Count the cells for viability and transfer to a sterile shaker flask at a minimum seeding density of 2 x 10^5 cells/mL.
4. Pass the cells using standard cell culture techniques.

Characteristics

Appearance
Off-white free-flowing powder

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

SAFC Biosciences warrants to the purchaser for a period of one year from date of delivery that this product conforms to its specifications. Other terms and conditions of this warranty are contained in SAFC Biosciences' written warranty, a copy of which is available upon request. ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING THE IMPLIED WARRANTY OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE, ARE EXCLUDED. In no case will SAFC Biosciences be liable for any special, incidental, or consequential damages arising out of this product or the use of this product by the customer or any third party based upon breach of warranty, breach of contract, negligence, strict tort, or any other legal theory. SAFC Biosciences expressly disclaims any warranty against claims by any third party by way of infringement or the like. THIS PRODUCT IS INTENDED FOR PURPOSES DESCRIBED ONLY AND IS NOT INTENDED FOR ANY HUMAN OR THERAPEUTIC USE.

Additional Terms and Conditions are contained in the product Catalog, a copy of which is available upon request.

Issued September 2006 P24651
0805 0905 0406

www.safcbiosciences.com

United States

SAFC Biosciences, Inc.
13804 W. 107th Street
Lenexa, Kansas 66215
USA
Phone +1 913-469-5580
Toll free-USA 1 800-255-6032
Fax +1 913-469-5584
E-mail info-na@sial.com

Europe

SAFC Biosciences Ltd.
Smeaton Road, West Portway
Andover, Hampshire SP10 3LF
UNITED KINGDOM
Phone +44 (0)1264-333311
Fax +44 (0)1264-332412
E-mail info-eu@sial.com

Asia Pacific

SAFC Biosciences Pty. Ltd.
18-20 Export Drive
Brooklyn, Victoria 3025
AUSTRALIA
Phone +61 (0)3-9362-4500
Toll free-AUS 1 800-200-404
Fax +61 (0)3-9315-1656
E-mail info-ap@sial.com