SAFC Biosciences™
Accelerate Success™

Product Information

EX-CELL™ 302 Serum-Free Medium for CHO Cells

without L-glutamine, without sodium bicarbonate

CATALOG NO. 24324C

Description

EX-CELL™ 302 is a serum-free dry powder medium, which has been specifically developed for the long-term growth of transformed Chinese Hamster Ovary (CHO) cells in suspension for the expression of antibodies or protein products. EX-CELL™ 302 is an appropriate medium for use with the DHFR$^-$ or Glutamine Synthetase, or the GS System™, because it does not contain hypoxanthine, thymidine or L-glutamine. If EX-CELL™ 302 is to be used with CHO cells transformed using the GS selection system the media needs to be supplemented with GS Supplement 50X (Catalog No. 58672C) for the additional amino acids and nucleosides. For use with DHFR$^-$ selection system, we recommend the addition of 4 mM L-glutamine for optimal growth.

Due to current regulatory concerns about the sources of raw materials, EX-CELL™ 302 was developed using only recombinant human proteins that have molecular weights less than 10 kD. The total protein concentration found in EX-CELL™ 302 is less than 1 mg/L. Pluronic® F68 has been added at a final concentration of 0.1% to EX-CELL™ 302 to protect against shear damage in sparged bioreactor systems.

Catalog No. 24324C replaces Catalog No. 24312 and includes an alternate source of soy hydrolysate to that found in the original EX-CELL™ 302 formulation. With more consistent performance and improved filtration characteristics, the alternate hydrolysate will improve the overall performance and consistency of EX-CELL™ 302. Comparability testing utilizing the previous soy hydrolysate and the replacement hydrolysate demonstrated comparable growth-promoting characteristics.

Formulation

The formulation for EX-CELL™ 302 is proprietary to SAFC Biosciences. For additional information please call our Technical Services department.

Precautions

Use aseptic technique when handling or supplementing this medium after filtration. 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.

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

EX-CELL™ 302 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 21.21 g/L of EX-CELL™ 302 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 21.3 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.
7. 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 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 protected from light at 2 to 8 C.

Methods for Use

Adaptation

Mammalian cells can be adapted to serum-free conditions by direct adaptation from serum-containing media or 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 media supplemented with serum.

Gradual Weaning to Serum-Free Media (Recommended):

1. Passage cells from the serum-supplemented medium directly into EX-CELL™ 302 + 5% gamma irradiated Fetal Bovine Serum (FBS) (Catalog No. 12107C) using normal seeding densities.
2. Once confluent (80 - 90%), rinse with 20 mL of Dulbecco's Phosphate Buffered Saline (DPBS Modified) (Catalog No. 59321C) and trypsinize with 5 mL of trypsin. Add 15 mL of EX-CELL™ 302 + 2% FBS. Obtain a cell count and pellet the cells by centrifugation.
3. Passage cells into a 75 cm^2 flask containing 30 mL EX-CELL™ 302 + 2% FBS at a density of 1 x 10^5 cells/mL.
4. Allow cells to reach 80 - 90% confluency. Retain any detached cells. Observe viability of detached cells. If detached cells are not viable, discard. If viable, reserve and combine with trypsinized cells.
5. Trypsinize cells as in Step 2, reducing the amount of trypsin to 2 mL. Inactivate trypsin with EX-CELL™ 302 + 1% FBS. Centrifuge.
6. Resuspend cells in EX-CELL™ 302 + 1% FBS at a density of 2 x 10^5 cells/mL.
7. Observe cells daily. Gently rap the flask against the palm of the hand to detach cells, return to the incubator until cells reach 80 - 90% confluency (~ 4 - 5 days).
8. Once confluent, retain detached cells and rap flask 2 - 3 times on the palm of the hand or a padded surface. Avoid causing bubbles. Triturate cells to further remove cells from the flask and to break up clumping cells. Obtain a cell count and pellet the cells by centrifugation.
9. Resuspend cells at a density of 8 x 10^5 cells/mL in 75 cm^2 flasks containing EX-CELL™ 302 without serum until cells reach a density of 1.5-2 x 10^6 cells/mL (~ 3 - 4 days).
10. Triturate cells to break up clumps. Obtain a cell count and pellet the cells by centrifugation.
11. Resuspend in EX-CELL™ 302 at a density of 8 x 10^5 cells/mL. Incubate until cells reach a density of 1.5-2 x 10^6 cells/mL. Take note of cell clump size (number of cells/clump).
12. Passage cells as outlined in Steps 9 - 11.
13. Repeat Step 12 except seed 250 mL shaker flasks at 6 x 10^5 cells/mL in 60 mL at 60 rpm.
14. After the cultures have been established in EX-CELL™ 302, passage the cells for an additional 2 passages in 250 mL shaker flasks.
15. Cells can now be scaled up as necessary using standard procedures and densities.

NOTES: Adjust protocol as necessary to ensure proper adaptation to shakers. Start with shaker speed of 60 rpm and adjust over the next few passes.

If cell clumping is extensive, select for single cells by letting the large clumps settle to the bottom of the flask and pipetting the single cells for passage. Be sure to count total cells and then count the retained single cell suspension to ensure proper seed density.

Always centrifuge cells when passing during the adaptation.

Culture density should reach 2-3 x 10^6 cells/mL over 3 - 4 days.

Direct Adaptation to Serum-Free Media:

1. Passage the cells into pre-warmed (37 C) EX-CELL™ 302 at 1.5-2X the recommended seeding density (2-5 x 10^5 cells/mL).
2. Refeed the culture after 48 hours with a 100% exchange of fresh EX-CELL™ 302.
3. Allow the cultures to achieve a minimum density of 1 x 10^6 cells/mL before subculturing.
4. Subculture into fresh EX-CELL™ 302 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, 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™ 302 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™ 302 medium, 45% spent medium 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™ 302 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™ 302 medium.
5. Count the cells for viability and transfer to a sterile shaker flask at a seeding density of 6 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
Off-white free-flowing powder

Bioburden
≤ 500 CFU/100 mL

Endotoxin
≤ 10.0 EU/mL

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 P24324
0104 0805 0905 0106 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