

Product Information

EX-CELL™ 420 Serum-Free Medium for Insect Cells

with L-glutamine, without sodium bicarbonate

CATALOG NO. 24420C

Description

EX-CELL™ 420 is a complete dry powder medium developed and optimized for the serum-free growth of Sf9 and Sf21 insect cell lines. Cells can be subcultured directly into EX-CELL™ 420 from serum-free or serum-supplemented media with no adaptation period required. Cultures in EX-CELL™ 420 routinely exceed cell densities of 1×10^7 cells/mL with greater than 95% viability. Suspension cultures can be maintained, without refeeding, for more than 10 days with high cell density and high viability. Sf9 and Sf21 cells have been carried for more than 20 passages in EX-CELL™ 420 with no loss of viability. Protein expression and virus production are improved over serum-containing media.

Formulation

The formulation for EX-CELL™ 420 medium 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 precipitates. 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™ 420 is formulated with 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 30.78 g/L EX-CELL™ 420 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.

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4. Adjust the osmolality to 370 - 390 mOsm/kg H₂O with the amount of sodium chloride per liter specified on the label or Certificate of Analysis.
5. Add 0.35 g/L of sodium bicarbonate (Catalog No. 90421C) or 4.7 mL/L of sodium bicarbonate solution 7.5% (Catalog No. 59221C). Mix until dissolved.
5. While mixing the solution, adjust the pH to 6.2 ± 0.2 using KOH 1N or HCl 1N. Add cell culture grade water to bring the medium to final volume.
6. 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 μ m. For larger volumes, a low-protein binding 0.45 μ m pre-filter is recommended. To minimize CO₂ 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 CO₂ gas.
NOTE: Other supplements, such as antibiotics or L-glutamine, 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

Insect cells that have been grown in a conventional serum-supplemented medium can be readily grown in EX-CELL™ 420 with little or no adaptation. During adaptation, growth rates will usually be somewhat slower than normal expected rates.

Culture Techniques

Insect cells are normally grown at 27 C in an air atmosphere, as CO₂ is not required to maintain an appropriate pH. Exposure to temperatures above 30 C results in rapid deterioration of the culture. Medium should be stored at 2 to 8 C, protected from light. Allow medium to warm to room temperature prior to use.

Many insect cells do not adhere to monolayer substrates as well as mammalian cells. The use of trypsin, collagenase or other standard techniques for dissociating monolayers is not recommended. Cells can be dislodged from the substrate with a gentle stream of medium or by sharply rapping the flask against the palm of the hand.

Monolayer cultures can be used to initiate suspension cultures. Harvest the cells and suspend them in fresh medium at $2-5 \times 10^5$ cells/mL. During adaptation to suspension, cultures in EX-CELL™ 420 may form clumps. These aggregates can be disrupted by trituration with a Pasteur pipet. Alternatively, single cell suspensions can be selected during subculture by allowing the heavier clumps to settle and using only the single cells remaining in suspension for the seed.

Once fully adapted, the cells should be passed every 3 - 4 days at a seeding density of at least $2-4 \times 10^5$ cells/mL in shaker or spinner flasks. Seed 50 mL of cell suspension in 125 mL shaker flasks and 100 mL of cell suspension in 250 mL shaker flasks. Shaker speed should be 100 - 120 rpm and spinner speed should be 60 - 75 rpm.

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. For successful results, care must be taken when subculturing cells. 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.

Cryopreservation

Freezing:

Cells can be frozen in EX-CELL™ 420 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™ 420 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 5×10^6 to 1×10^7 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™ 420 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™ 420 medium.
5. Count the cells for viability and transfer to a sterile shaker flask at a seeding density of 2-4 x 10⁵ cells/mL.
6. When the culture has reached a density of 1 x 10⁶ cells/mL, passage the cells using standard cell culture techniques.

Characteristics

Appearance

Off-white free flowing powder

Bioburden

≤ 500 CFU/100 mL

Endotoxin

≤ 20.0 EU/mL

Osmolality (as supplied)

Refer to Certificate of Analysis

pH (as supplied)

Refer to Certificate of Analysis

Solubility

Soluble

The ability of this medium to support cells in culture was assessed using Sf9 cells in suspension culture. Tests were conducted in parallel with a validated control.

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.

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Issued September 2006 P24420
1103 0805 0905 0406

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