SAFC Biosciences™
Accelerate Success™

Product Information

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

with L-glutamine

CATALOG NO. 14420C

Description

EX-CELL™ 420 is a complete 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 without adaptation. Cultures in EX-CELL™ 420 routinely reach cell densities greater than 1 x 10^7 cells/mL with greater than 95% viability. Suspension cultures can be maintained, without refeeding, for more than 10 days. 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 is proprietary to SAFC Biosciences. For additional information 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 medium at 2 to 8 C, protected from light. Do not use after expiration date.

Indications of Deterioration

Medium should be clear and free of particulates and flocculent material. Do not use if 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™ 420 is formulated with L-glutamine. Other 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 supplements.

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 approximately 27 C in an air atmosphere, as CO_2 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.

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

Monolayer cultures can be used to initiate suspension cultures. Harvest the cells and suspend them in fresh medium at 2-5 x 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 x 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 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™ 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 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
Clear yellow solution

Endotoxin
≤ 20.0 EU/mL

Osmolality (as supplied)
370 - 390 mOsm/kg H_2O

pH (as supplied)
6.0 - 6.4

Sterility
No microbial growth detected

The ability of this medium to support cells in culture was assessed using established cell lines. Tests were conducted in parallel with a validated control. Results are available on request.

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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