

Product Information

EX-CELL™ 405 Serum-Free Medium for Insect Cells

with L-glutamine, without sodium bicarbonate

CATALOG NO. 24405C

Description

Baculoviruses are powerful expression systems for production of recombinant proteins. The most well known and highly investigated baculovirus vector expression system (BEVS) uses *Spodoptera frugiperda* cells (especially Sf9) as the host cell of choice for infection with recombinant *Autographa californica* nuclear polyhedrosis virus for heterologous protein expression. Recent investigations have shown that other cell lines may be better expression substrates than spodopteran cells. The BTI-TN-5B1-4 clone of *Trichoplusia ni*, commonly referred to as High Five™, is reported to be a superior host substrate for the expression of selected recombinant proteins using BEVS. This cell line portrays strong adherent characteristics, but can be adapted to suspension growth.

EX-CELL™ 405 is a serum-free dry powder medium for optimal growth and viability of BTI-TN-5B1-4 (Tn5, High Five™) cells. In addition, EX-CELL™ 405 supports recombinant protein production in High Five™ cells that in many instances are reported to achieve higher levels of expression than Sf9 cells. EX-CELL™ 405 is a complete medium. No protein supplements need to be added to this medium prior to use.

Formulation

The formulation for EX-CELL™ 405 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™ 405 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.

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2. Slowly add 42.71 g/L of EX-CELL™ 405 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 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 μm . For larger volumes, a low-protein binding 0.45 μm 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™ 405 with little or no adaptation. During adaptation, growth rates will usually be somewhat slower than normal expected rates.

Culture Techniques

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™ 405 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™ 405 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™ 405 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™ 405 medium.
5. Count the cells for viability and transfer to a sterile shaker flask at a seeding density of $2-4 \times 10^5$ cells/mL.
6. When the culture has reached a density of 1×10^6 cells/mL, passage the cells using standard cell culture techniques.

Characteristics

Appearance

Off-white free flowing powder

Bioburden

≤ 500 CFU/100 mL

Endotoxin

≤ 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 High Five™ 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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High Five™ is a trademark of Invitrogen Corporation.

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