

Product Information

EX-CELL™ HeLa Serum-Free Medium for HeLa Cells

without L-glutamine

CATALOG NO. 14591C

Description

EX-CELL™ HeLa is an animal-protein free, serum-free liquid medium developed for the long-term growth of HeLa cells in suspension culture. The HeLa cell line is a clonal derivative of the parent HeLa cell line and is capable of growth in suspension culture. HeLa suspension cultures can be subcultured directly into EX-CELL™ HeLa from serum-supplemented or serum-free media. Suspension cultures in EX-CELL™ HeLa have been carried for more than 25 passages with no loss of growth or viability.

Catalog No. 14591C replaces Catalog No. 14590 and includes an alternate source of soy hydrolysate to that found in the original EX-CELL™ HeLa formulation. The new formulation also contains a synthetic D-galactose, which replaces bovine milk-derived D-galactose. The alternate hydrolysate offers more consistent performance and improved filtration characteristics, which will improve the overall performance and consistency of EX-CELL™ HeLa. In both cases, comparability testing utilizing the previous components and the replacement components demonstrated comparable growth-promoting characteristics.

Formulation

The formula for EX-CELL™ HeLa 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 liquid medium at 2 to 8 C, protected from light. Do not use after the expiration date.

Indications of Deterioration

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

EX-CELL™ HeLa is formulated without L-glutamine. Prior to use, this medium should be supplemented with 6 mM L-glutamine by adding 30 mL/L of a 200 mM solution (Catalog No. 59202C). SAFC Biosciences recommends L-glutamine supplementation of the working volume only. SAFC Biosciences also recommends the supplementation of 10 - 25 mM HEPES buffer in applications outside of a pH-controlled environment (such as stationary T-flasks, roller bottles and spinner flasks) by supplementing with 10 - 25 mL/L of HEPES Solution 1M (Catalog No. 59205C). 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 supplement.

Methods for Use

Adaptation

HeLa cells that have been grown in a conventional serum-supplemented medium can be readily grown in EX-CELL™ HeLa with little adaptation. Adaptation to EX-CELL™ HeLa requires healthy, viable cultures in mid-logarithmic growth phase.

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1. Subculture the cells from serum-supplemented medium to EX-CELL™ HeLa supplemented with 6 mM L-glutamine at a minimum seeding density of 3 x 10⁵ cells/mL in shaker flasks.
2. Incubate the flasks at 37 C in a humidified incubator with 5% CO₂. Maintain the orbital shaker speed between 120 - 150 rpm.
3. Continue to subculture cells in EX-CELL™ HeLa every 3 - 4 days, using the above seeding density.
4. Allow the cells to adapt to EX-CELL™ HeLa for an additional 4 - 6 passages. Cells are considered fully adapted to EX-CELL™ HeLa when growth rates return to normal and viabilities are above 95%.

Culture Techniques

HeLa cells are normally grown at 37 ± 1 C and 5% CO₂. 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 3 x 10⁵ 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 120 - 150 rpm.

When passing the cells, medium 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:

HeLa cells can be frozen in EX-CELL™ HeLa without the reintroduction of serum. However, it is necessary to handle the cells gently and freeze the cells under carefully controlled conditions. The addition of D-sucrose to the freezing medium is recommended at a final concentration of 0.1%. Prepare a 10% (100X) solution of D-sucrose in high-quality water and sterile filter through a 0.2 µm membrane filter.

1. Choose cultures in logarithmic growth with viabilities above 90%.
2. Prepare a freezing medium consisting of 45% cold EX-CELL™ HeLa medium, 45% spent medium, 9.9% dimethyl sulfoxide (DMSO) and 0.1% D-sucrose.
3. Centrifuge the cells at 200 g for 5 minutes. Remove the supernatant and prepare the freezing medium.
4. Resuspend the cells in the freezing medium at 1 x 10⁷ 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™ HeLa 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™ HeLa medium.
5. Count the cells for viability and transfer to a sterile shaker flask at a seeding density of 3 x 10⁵ cells/mL.
6. Pass the cells using standard cell culture techniques.

Characteristics

Appearance

Clear yellow solution

Endotoxin

Refer to Certificate of Analysis

Osmolality (as supplied)

260 - 300 mOsm/kg H₂O

pH (as supplied)

7.0 - 7.4

Sterility

No microbial growth detected

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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