SAFC Biosciences™
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Product Information

EX-CELL™ 293 Serum-Free Medium for HEK 293 Cells

without L-glutamine

CATALOG NO. 14571C

Description

EX-CELL™ 293 is an animal-protein free, serum-free liquid medium developed for the long-term growth of Human Embryonic Kidney 293 (HEK 293) and related cells. The cells, in a suspension culture, can be subcultured directly into EX-CELL™ 293 from serum-supplemented media with little or no adaptation. Suspension cultures can be maintained, without refeeding for about 10 days and can be carried for more than 20 passages with no loss of viability.

Catalog No. 14571C replaces Catalog No. 14570 and includes an alternate source of soy hydrolysate to that found in the original EX-CELL™ 293 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™ 293. In both cases, comparability testing utilizing the previous components and the replacement components demonstrated comparable growth-promoting characteristics.

Formulation

The formula for EX-CELL™ 293 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 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 particulate and flocculent material. Do not use if liquid medium is cloudy or contains precipitates. Other evidence of deterioration may include color change, pH shift and degradation of physical or performance characteristics.

Preparation Instructions

EX-CELL™ 293 is formulated with sodium bicarbonate and 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

HEK 293 cells that have been grown in a conventional serum-supplemented medium can be readily grown in EX-CELL™ 293, with little or no adaptation. Adaptation to EX-CELL™ 293 requires healthy, viable cultures in mid-logarithmic growth phase. During adaptation, growth rates will usually be somewhat slower than normal expected rates.

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Contact Technical Service for information regarding direct adaptation to another serum-free medium. Alternatively, if adapting from another serum-free medium, we recommend cultures be adapted to Dulbecco's Modified Eagle's Medium/High Modified (DMEM/High) (Catalog No. 51444C) supplemented with 6 mM L-glutamine and 5% gamma irradiated Fetal Bovine Serum (FBS) (Catalog No. 12107C) for at least 3 passages prior to adapting the HEK 293 cells to EX-CELL™ 293. The adherent cells in DMEM/High with serum can be detached by trypsin. Inactivate the trypsin with media containing 5% FBS.

1. Subculture the cells from serum-supplemented medium to EX-CELL™ 293 using standard trypsinization techniques when cultures reach 100% confluence.
2. Inactivate the trypsin with media containing 5% FBS. Using low-speed centrifugation, pellet the cell suspension at 200 *g* for 5 minutes and carefully decant the supernatant without disturbing the cell pellet.
3. Resuspend the cells in EX-CELL™ 293 medium at a density of 6 x 10^5 cells/mL in shaker flasks.
4. Allow the cells to adapt to EX-CELL™ 293 for an additional 4 - 6 passages. Cells are considered fully adapted to EX-CELL™ 293 when growth rates return to normal densities and viabilities are above 95%.
5. Continue to subculture cells in EX-CELL™ 293 at a density of at least 4 x 10^5 cells/mL into shaker or spinner flasks.

Culture Techniques

HEK 293 cells are normally grown at 37 ± 1 C and 5 - 10% CO_2. Allow the medium to warm to room temperature prior to use. Once fully adapted, the cells should be passed at a seeding density of at least 4 x 10^5 cells/mL in shaker or spinner flasks. Seed 30 mL of cell suspension in 125 mL shaker flasks and 60 mL cultures in 250 mL shaker flasks. Shaker speed should be 100 - 120 rpm and spinner speed should be 60 - 75 rpm.

When passing the cells, carry over should not exceed 25% of the final volume. If carry over 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™ 293 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™ 293 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™ 293 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™ 293 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. 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 H_2O

pH (as supplied)
7.0 - 7.4

Sterility
No microbial growth detected

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