

Product Information

EX-CELL™ 293

Serum-Free Medium for HEK 293 Cells

with L-glutamine, without sodium bicarbonate

CATALOG NO. 24571C

Description

EX-CELL™ 293 is an animal-protein free, serum-free dry powder 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. 24571C replaces Catalog No. 24570 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 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 formulation 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 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 precipitate. Other evidence of deterioration may include color change, pH shift and 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™ 293 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 size mixing vessel. Water temperature should be 20 to 30 C.

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

2. Slowly add 21.58 g/L of EX-CELL™ 293 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. Adjust the pH to 5.0 with HCl 1N, mix to equilibrate and adjust the pH to 6.8 with NaOH 1N (Catalog No. 59223C).
5. Add 1.8 g/L of sodium bicarbonate (Catalog No. 90421C) or 24 mL/L of sodium bicarbonate solution 7.5% (Catalog No. 59221C). Mix until fully dissolved.
6. While mixing the solution, adjust the pH to 6.9 - 7.1 using NaOH 1N or HCl 1N. The pH of this medium usually rises 0.1 - 0.2 units during the filtration. For most applications, the optimal pH of the filtered medium is 7.0 - 7.4.
7. Add cell culture grade water to the solution to bring it to final volume. To avoid fluctuation in pH, keep the vessel closed until the medium is filtered.
8. To sterilize the medium, first use a low protein-binding 0.45 µm pre-filter, followed by a sterilizing low protein-binding membrane filter with a pore size of 0.22 µm. 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. SAFC Biosciences 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). Storage conditions and shelf life of the supplemented product may be affected by the nature of the supplements.

9. Dispense medium into sterile containers using aseptic technique. Store liquid medium protected from light at 2 to 8 C.

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.

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 6×10^5 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 4×10^5 cells/mL into shaker or spinner flasks.

Culture Techniques

HEK 293 cells are normally grown at 37 ± 1 C and 5 - 10% CO₂. 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 4×10^5 cells/mL in shaker or spinner flasks. Seed 30 mL 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 5×10^6 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™ 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 6×10^5 cells/mL.
6. Pass the cells using standard cell culture techniques.

Characteristics

Appearance

White free-flowing powder

Bioburden

Refer to Certificate of Analysis

Endotoxin

Refer to Certificate of Analysis

Osmolality (as supplied)

Refer to Certificate of Analysis

pH (as supplied)

Refer to Certificate of Analysis

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www.safcbiosciences.com

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