SAFC Biosciences™
Accelerate Success™

Product Information

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

with L-glutamine, without sodium bicarbonate

CATALOG NO. 24581C

Description

EX-CELL™ MDCK is an animal-protein free, serum-free dry powder medium developed for the long-term growth of Madin Darby Canine Kidney (MDCK) and related cells. The cells, in an attachment culture, can be subcultured directly into EX-CELL™ MDCK from serum-supplemented media without adaptation. Cell densities and doubling times achieved under serum-free conditions are comparable to those achieved in a serum-supplemented culture.

Catalog No. 24581C replaces Catalog No. 24580 and includes an alternate source of soy hydrolysate to that found in the original EX-CELL™ MDCK 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™ MDCK. In both cases, comparability testing utilizing the previous components and the replacement components demonstrated comparable growth-promoting characteristics.

Formulation

The formulation for EX-CELL™ MDCK 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™ MDCK 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.
2. Slowly add 21.58 g/L of EX-CELL™ MDCK 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.

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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 CO_2 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 CO_2 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

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

1. Subculture the cells from serum-supplemented medium to EX-CELL™ MDCK using standard trypsinization techniques when cultures reach 100% confluence.
2. Inactivate the trypsin with media containing 5% gamma irradiated Fetal Bovine Serum (FBS) (Catalog No. 12107C) or soybean trypsin inhibitor (0.1%). 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™ MDCK medium at a density of 1-2 x 10^5 cells/cm^2.
4. Allow the cells to adapt to EX-CELL™ MDCK for an additional 4 - 6 passages. Cells are considered fully adapted to EX-CELL™ MDCK when growth rates return to normal densities and viabilities are above 95%.
5. Continue subculturing cells in EX-CELL™ MDCK at a density of at least 1 x 10^5 cells/cm^2.

Culture Techniques

MDCK 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 1 x 10^5 cells/cm^2.

Cells grown in medium without serum are extremely fragile and sensitive to the trypsin used to remove adherent cells from a substrate. For successful results, care must be taken when subculturing cells.

Normal trypsin concentrations may be used, but incubations should be carried out at 4 C, and exposure time should be minimal. SAFC Biosciences recommends the use of a soybean trypsin inhibitor (0.1%) or sedimentation by centrifugation to remove the trypsin. Soybean trypsin inhibitor should be used with caution, as it is toxic to many cells. Cells may also be dislodged by NO-ZYME™ (Catalog No. 59226C), a non-enzymatic dissociating agent.

In addition, standard techniques of centrifugation must be modified to include low-speed centrifugation to prevent damage to cells that have been propagated in a serum-free medium.

Cryopreservation

Freezing:

Cells can be frozen in EX-CELL™ MDCK without the reintroduction of serum.

1. Choose cultures in logarithmic growth with viabilities above 90%.
2. Prepare a freezing medium consisting of 90% cold EX-CELL™ MDCK medium and 10% dimethyl sulfoxide (DMSO).
3. Using standard trypsinization techniques, collect and centrifuge the cells at 200 *g* for 5 minutes. Remove the supernatant without disturbing the cell pellet.
4. Resuspend the cells in the freezing medium at 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™ MDCK 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™ MDCK medium.
5. Count the cells for viability and transfer to a sterile tissue culture flask at a seeding density of 2-3 x 10^5 cells/cm^2.
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

Warranty, Limitation of Remedies

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Additional Terms and Conditions are contained in the product Catalog, a copy of which is available upon request.

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