SAFC Biosciences™
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Product Information

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

without L-glutamine

CATALOG NO. 14581C

Description

EX-CELL™ MDCK is an animal-protein free, serum-free liquid 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. 14581C replaces Catalog No. 14580 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

EX-CELL MDCK should be stored at 2 to 8 C, protected from light. Do not use after the expiration date.

Indications of Deterioration

EX-CELL™ MDCK 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™ MDCK 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

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.

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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
Clear yellow solution

Endotoxin
Refer to Certificate of Analysis

Osmolality (as supplied)
260 - 300

pH (as supplied)
7.0 - 7.4

Sterility
No microbial growth detected

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