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ProductInformation

MEDIUM SYSTEM FOR MDBK CELLS

Product Nos. **M 3553 AND M 0682**
Storage Temperature 2-8 °C

Product Description
The MDBK serum-free system has been designed for rapid growth and maintenance of MDBK cell cultures. The system consists of two media: (1) MDBK-GM (Product No. M 3553), a serum-free medium containing 150 µg/ml of animal-derived protein and designed for rapid cell growth and (2) MDBK-MM (Product No. M 0682), a protein-free medium supplemented with recombinant peptides that is designed to maintain high cell densities for an extended period of time. Mixtures of different ratios of both growth and maintenance media allow for system flexibility. The system has been designed to allow for customized applications and is intended for use with MDBK cells only.

Precautions and Disclaimer
For R&D use only. Not for drug, household or other uses. MSDS available upon request or at sigma-aldrich.com. Cytodex is a trademark of Amersham Biosciences.

Preparation Instructions
MDBK-GM and MDBK-MM are supplied as sterile liquids. L-Glutamine (Product No. G 7513) supplementation is required before use. MDBK-GM requires the addition of 16.5 ml of 200 mM L-glutamine per liter of medium, while MDBK-MM requires the addition of 20 ml of 200mM L-glutamine per liter.

Storage/Stability
Products are stable for a year if stored at 2-8 °C and protected from light until the indicated expiration date on the label.

Procedure
Rapid growth without weaning or lag time is obtained by using MDBK-GM for 2-5 days and then switching to MDBK-MM. Under these conditions, flasks inoculated at 10,000 cells/cm^2 or roller bottles inoculated at 25,000 cells/ cm^2 expands to 500,000 cells/cm^2 and can be maintained for at least two weeks in either static or dynamic cultures. MDBK-GM will not maintain non-dividing cultures, even with frequent medium changes.

The maintenance medium can be introduced to the culture system between days 3 and 5 to replace the growth medium. Therefore, frequency of medium change and amount of protein used in the system can be modified to optimize performance. In addition, a 1:1 mixture of MDBK-GM and MDBK-MM can be used to create a “single-medium system” to grow and maintain cells. Utilizing this system, the log phase can be extended from 5 to 9 days with more time for sub-culturing. Culture viability in high-density cultures should be monitored by observing change in medium color and measuring both glucose and lactate levels. When the glucose level falls below 1.0 g/L and /or the lactate level rises to 2.0 g/L, medium should either be replaced or cultures terminated.

Sub-culture
Cells should only be sub-cultured when in the log phase. Non-dividing cultures maintained in MDBK-MM are not suitable for successive passages. MDBK cells undergo at least four passages in MDBK-GM without changes in doubling time. The recommended minimum inoculation is 10,000 cells/cm^2. Due to the low level of protein in the growth medium, care must be exercised to protect cells from prolonged trypsinization. The following protocol has been used successfully in flasks:

1. Cultures are briefly washed with 0.2 ml/cm^2 DPBS containing calcium and magnesium (Product No. D 8662).
2. Cold Trypsin-EDTA (Product No. T 3924) at a volume of 0.2 ml/cm^2 is then added to the flask and allowed to cover the culture surface for 30 to 60 seconds at room temperature.
3. After removal of the Trypsin-EDTA, cultures are incubated at 37 °C until cells become rounded and easily dislodged from the surface by gently rapping the flask (approximately 1-2 minutes).
4. Cells are resuspended in a 10 µg/ml solution of soybean trypsin inhibitor (Product No. T 6414), at a volume of 0.2 ml/cm^2 of original surface area.
5. Cells are pelleted at 1000 rpm for 5 minutes, trypsin inhibitor removed, and cells resuspended in at least 0.1 ml of DPBS/cm^2 of original surface area.

6. Cells are then plated in pre-warmed growth medium at approximately 100 □l of the cell suspension to 1 ml of medium. Changes may be required for different culture conditions. For example, long-term confluent cultures require a larger volume of trypsin-EDTA and longer trypsinization time.

Caution: The use of more vigorous trypsinization procedures or failure to neutralize excess trypsin can result in culture failure.

Roller bottle cultures

MDBK cultures have been successfully established and maintained in roller bottles. Cells are inoculated at a density of 25,000 cells/cm^2 in a volume of 0.2 ml/cm^2 of pre-warmed (37 °C) medium. Initial rotational speed should not exceed 0.2-0.3 rpm. After 12 to 24 hours, cells are firmly attached to the surface and speed should be increased to 0.5 rpm. Medium changes should follow the schedule previously described.

Spinner flasks cultures with microcarrier beads

In our studies, 100 ml spinner flasks containing 75 ml of medium and 4 mg/ml beads were inoculated with 25,000 cells/mg of beads. MDBK cells were observed to attach to Cytodex™ 1 and 3 and microcarrier beads from Solohill Labs, Inc (Ann Arbor, MI). Refer to manufacturer's guidelines for microcarrier handling. The use of sufficient cell inoculum to populate all the beads is vital because cells were not observed to migrate from one bead to another.

Cryopreservation

MDBK cells grown in MDBK-GM have been successfully frozen in liquid nitrogen and recovered. Actively dividing cells are trypsinized according to the protocol previously described, pelleted by centrifugation and resuspended at a concentration of 1 x 10^6 cells/ml in serum-free cell freezing medium (Product No. C 6295). Cells are frozen in liquid nitrogen according to standard procedures. Cells are recovered by rapid thawing and centrifuging at 500 rpm for 5 minutes. The pellet is resuspended in a pre-warmed "recovery" medium and seeded at a density of 40,000 cells/ cm^2. Cultures are carried for 3 days in "recovery" medium before the addition of MDBK-GM.

Product Profile

pH at RT	7.0 – 7.6
Osmolality	285 - 315 mOsm/kg H_2O

References

1. Cruz, H.J., et al. (1997). Cell-dislodging methods under serum-free conditions. Applied Microbiology Biotechnology, **47**:482-488.
2. Leighton, J., et al. (1970). A cell line from normal dog kidney (MDCK) exhibiting qualities of papillary adenocarcinoma and renal tubular epithelium. Cancer, **26**:1022-1028.
3. Noe, W., et al. (1994). Optimization of vaccine production for animal health. Cytotechnology,
4. **15**:169 -176.
5. Taub, M., et al. (1979). Growth of Madin-Darby canine kidney epithelial cell (MDCK) line in hormone-supplemented, serum-free medium. Proc. Natl. Acad. Sci. USA, **76**: 3338-3342.

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