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# ProductInformation

**MEDIUM FOR MDCK CELLS**
Product Number **M 3803 AND M 3678**
Storage Temperature 2-8 °C

## Product Description

Sigma media M 3803 (MDCK Low-protein Medium) and M 3678 (MDCK Protein-free Medium) are formulated for the growth of Madin-Darby canine kidney cells (MDCK). The low-protein medium has a protein content of 100 µg/ml (albumin) while the protein-free medium is devoid of all animal-derived protein.

## Precautions and Disclaimer

For laboratory use only. Not for drug, household or other uses. Products are expiration dated. Sigma is not responsible for products whose expiration date has passed. Exercise appropriate care when handling chemicals. MSDS available.

## Preparation Instructions

L-Glutamine (Sigma product No. G 7513) supplementation (4mM) is required before use: MDCK-LPM and MDCK-PFM require the addition of 16.5ml/L.

## Storage/Stability

This medium is stable, when stored 2-8 °C and protected from light, until the indicated expiration date on the label.

## Procedures

### *Handling and Culture of cells*

MDCK cells can be grown in either M 3803 or M 3678 without adaptation from a serum-supplemented source while maintaining their epithelial-like morphology. MDCK cells have been subcultured in these media up to four passages without changes in population doubling level (PDL). Cultures can be initiated in the low-protein medium, which provides better attachment, and then switched to the protein-free formulation in which cells can be maintained in a protein-free environment.

Cultures reach optimum cell density between days 5 and 6 when inoculated at 20,000 cells/ $cm^2$. Higher cell densities are obtained by changing medium more often, i.e. every 3-4 days. All cells should be passed into

Because cells cultured in low-protein and protein-free media are very sensitive to mechanical damage, these media are best suited for static cultures or culture conditions with low shear forces. Roller bottle speed should be decreased (0.2 to 0.3 rpm) and low-protein medium used to allow for cell attachment. Conditions with higher shearing forces will require modifications to the formulations.

Because these media are serum-free, care must be exercised to protect the cells from prolonged trypsinization. The following trypsinization procedures will lead to high cell viability and increased plating efficiency. Cultures should be washed twice with calcium- and magnesium-free saline solution like Dulbecco's PBS (Sigma Product No. D 8537) or Hanks' BSS (Sigma Product No. H 6648). Trypsin-EDTA (Sigma Product No. T 3924) at a volume of approximately 0.2ml/$cm^2$ is dispersed over the entire surface area. Cultures should then be incubated for 2 to 3 minutes at 37 °C. Trypsin-EDTA is then removed and cultures re-incubated at $37^{O}$C until the cells become rounded and easily dislodged from the surface by gently rapping the flask (approximately 1-2 minutes). The trypsinized cell suspension is resuspended in a 10µg/ml solution of soybean trypsin inhibitor (Sigma Product No. T 6414), at a volume of 0.2ml/$cm^2$ of original surface area. The cells are pelleted at 500rpm for five minutes, trypsin inhibitor removed and cells resuspended in at least 0.1ml of Dulbecco's PBS/$cm^2$ of original surface area. Cells are then plated in pre-warmed growth medium at approximately 100 µl of cell suspension to 1 ml of medium.

### *Cryopreservation*

MDCK cells grown in low-protein and protein-free media can be successfully frozen in liquid nitrogen and recovered. Actively dividing cultures should be trypsinized, pelleted by centrifugation and resuspended at a concentration of $1X10^6$ cells/ml in a serum-free cryopreservation solution (Sigma Product No. C 6295). Cells are frozen in liquid nitrogen according to standard procedures.

Cells are recovered by rapid thawing and centrifuged at 1,000 rpm for 5 minutes. The pellet is resuspended in pre-warmed medium and seeded at a density of 40,000 cells/ $cm^2$. An equal volume of medium is added after 48 hours and complete medium change is performed on day 5.

**Product Profile**

M 3803

pH at room temperature 7.0 – 7.6

Osmolality 270 – 330 mOsm/kg $H_2O$

M 3678

pH at room temperature 7.0 – 7.6

Osmolality 285 – 315 mOsm/kg $H_2O$

**References**


1. Gaush, C.R., W.L. Hard and T.F. Smith (1966) Characterization of an established line of canine kidney cells (MDCK). Proc. Soc. Exp. Biolo. Med. 122:931-935.

2. McRoberts, J.A., M. Taub and M.H. Saier Jr. (1981) The Madin-Darby canine kidney (MDCK) cell line. In: Functionally Differentiated Cell Lines (G. Sata, ed.) pp. 117-139.

3. Taub, M., L. Chuman, M.H. Saier Jr., and G. Sato 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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