

Product Information

ADME/Tox Cell Lines Canine MDR1 Knockout, Human BCRP/OATP2B1 Knockin MDCKII Cells

Catalog Number **MTOX1302**

Storage Temperature $-130\text{ }^{\circ}\text{C}$ or below
in liquid nitrogen vapor phase

TECHNICAL BULLETIN

Product Description

MDCKII is a subclone derived from the heterogenous parent line MDCK (Madin-Darby canine kidney, ECACC Catalog No. 85011435). MDCK II cells, which predominate in later passages from MDCK, are reported to display electrical resistance of $100\text{ ohm}/\text{cm}^2$. This strain is thought to be derived from the distal tubule or collecting duct of the nephron. The cell line can be used as an experimental model to study the generation and maintenance of cell surface polarity in epithelial cells.

CRISPR technology is a fast and reliable way to manipulate the genome in a targeted fashion. CRISPR/Cas systems have evolved within bacterial and archaeal organisms as a defense against invading viruses and plasmids. Recently, the type II CRISPR/Cas system from the bacterium *Streptococcus pyogenes* has been engineered to function in eukaryotic cells using two molecular components: a single Cas9 protein and a non-coding guide RNA (gRNA).¹⁻⁷ The Cas9 endonuclease can be programmed with a single gRNA, directing a DNA double-strand break (DSB) at a desired genomic location. Similar to DSBs induced by zinc finger nucleases (ZFNs), the cell then activates endogenous DNA repair processes, either non-homologous end joining (NHEJ) or homology-directed repair (HDR), to heal the targeted DSB.

ATP-binding cassette (ABC) transporters are a family of transmembrane proteins that utilize ATP hydrolysis for translocation of substrates across membranes. ABC transporters are known to play a critical role in the development of multidrug resistance. Evaluation of membrane transporter pharmacology in drug disposition and drug-drug interactions (DDI) is critical to the pharmaceutical safety evaluations of new drug entities.

Selection of the targeted gene(s) was based on the considerable body of evidence supporting its crucial role in the development of multidrug resistance.⁸

Cell Line Description:

Parental Cell Line: MDCKII, ECACC Catalog No. 00062107.

Note: Please see 00062107 product datasheet from ECACC for additional information about the origin of this cell line. Cytogenetic information is based on initial seed stock at Sigma Life Science. Cytogenetic instability has been reported in the literature for some cell lines.

Components

This product contains 1 cryovial (2–6 million cells) of Canine MDR1 Knockout, Human BCRP and OATP2B1 Knockin MDCKII Cells. The vial contains sufficient cells to seed one 24 or 96 well Millicell® plate.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Precaution: It is recommended protective gloves and clothing always be used, and a full face mask always be worn when handling frozen vials. It is important to note some vials leak when submerged in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to the gas phase may result in the rapid expansion of the vessel, potentially blowing off its cap with dangerous force creating flying debris. At the time a cell line is ordered end users should also consider the culture conditions for the new cell line and make sure the appropriate measures are taken.

Storage/Stability

Upon receiving a shipment of frozen cells it is important the end user gives the shipment attention without delay. To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival continued storage of the frozen culture is necessary; the cells should be stored in liquid nitrogen vapor phase and not at $-70\text{ }^{\circ}\text{C}$. Storage at $-70\text{ }^{\circ}\text{C}$ will result in loss of viability.

Procedure

Protocol for Thawing and Seeding 24 Well or 96 well plates

Note: One cryovial contains enough cells to seed one plate.

Reagents and Equipment Required but Not Provided.

Note: Neither media nor supplements are supplied with the vials. These must be obtained prior to receiving the vials.

- Fetal Bovine Serum (FBS), Catalog No. F4135
- Minimum Essential Medium Eagle (MEM), Catalog No. M2279
- L-Glutamine solution, Catalog No. G7513
- Penicillin-Streptomycin, Catalog No. P4333
- Millicell 24-well plate assembly, Catalog No. PSRP010R5
- Millicell 24-well receiver tray, Catalog No. SPMW010R
- Millicell 96-well plate assembly, Catalog No. PSRP004
- Millicell 96-well receiver tray, Catalog No. SPMW004R

MDCKII Medium Preparation

Prepare MDCKII Medium by adding FBS, Catalog No. F4135, at a final concentration of 10% (v/v) in MEM, Catalog No. M2279. MEM is supplemented with L-Glutamine, Catalog No. G7513, to a final concentration of 2 mM. Penicillin-Streptomycin, Catalog No. P4333, is added to MEM at a final concentration of 1% (v/v). The MDCKII Medium is formulated for use with a 5% CO_2 in air atmosphere.

Thawing Cells and Seeding Plates

1. Pre-warm MDCKII Medium in a $37\text{ }^{\circ}\text{C}$ water bath.
2. Pipette 10 ml of pre-warmed MDCKII Medium into a sterile centrifuge tube.
3. Moisten an absorbent paper with 70% ethyl alcohol.
4. Remove cryovial from liquid nitrogen. Under a laminar flow hood, briefly twist the cap a quarter turn to relieve the internal pressure and then close again.
5. Quickly transfer the cryovial to a $37\text{ }^{\circ}\text{C}$ water bath. While holding the tip of the vial, gently agitate for 1–2 minutes, being careful not to allow water to penetrate the cap.
Note: Do not submerge cryovial completely.
6. Watch the cryovial closely. When just a small crystal of ice remains, remove it from the water bath.
7. Wipe the outside of the vial with absorbent paper moistened with 70% ethyl alcohol and place it under laminar flow hood.
8. Aseptically transfer the cell suspension to the centrifuge tube with pre-warmed MDCKII Medium and pellet cells by centrifugation at 800 rpm for 3 minutes.
9. Aspirate the MDCKII Medium from the tube, and re-suspend the cell pellet in the appropriate amount of pre-warmed MDCKII Medium (13 ml for 24 well plates or 11 ml for 96 well plates).
10. Mix cells thoroughly and transfer 500 μl per well (Millicell 24-well plate assembly, Catalog No. PSRP010R5) or 100 μl per well (Millicell 96-well plate assembly, Catalog No. PSRP004).
11. Add 25 ml of MDCKII medium (if using Millicell 24-well receiver tray, Catalog No. SPMW010R) or 5 ml of medium (if using Millicell 96-well receiver tray, Catalog No. SPMW004R) to the bottom receiver tray of plate assembly.
12. Place the plate assembly in a $37\text{ }^{\circ}\text{C}$, 5% CO_2 incubator. Incubate the plate assembly until the cells form a confluent monolayer, replacing the MDCKII Medium in both the wells and receiver tray every 3–4 days. Assays may be performed on post-confluent cells from days 2–5.

References

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2. Cong, L. et al., Multiplex genome engineering using CRISPR/Cas systems. *Science*, 2013; 339(6121) 819-23.
3. Friedland, A. E. et al, Heritable genome editing in *C. elegans* via a CRISPR-Cas9 system. *Nat. Methods*, 2013 Jun 30. doi: 10.1038/nmeth.2532.
4. Hwang, W.Y. et al., Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nat. Biotechnol.*, 2013; 31(3): 227-9.
5. Jinek. M. et al., A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, 2012; 337(6096): 816-21.
6. Jinek, M. et al., RNA-programmed genome editing in human cells. *Elife*, 2013; 2:e00471. doi: 10.7554/eLife.00471.
7. Mali, P. et al., RNA-guided human genome engineering via Cas9. *Science*, 2013; 339(6121):339(6121):8 23-6.
8. The International Transporter Consortium (2010 White Paper), Membrane transporters in drug development. *Nature Reviews Drug Discovery*, **9**, 215-236 (2010).
9. Chen, W. et al. in *Cell Culture Models of Biological Barriers In-Vitro Test Systems for Drug Absorption and Delivery*. (Lehr, C-M., ed.), Taylor & Francis, (New York, NY: 2002) pp. 143-163.

Additional product and technical information can be obtained at www.sigma.com.

MDCKII subclone was originally isolated by Daniel Louvard, Institut Curie, Paris France.
References: Hansson, G.C. et al., *EMBO*, 1986; 5, 483-489.
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