SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

ADME/Tox Cell Lines
Canine MDR1 Knockout, Human BCRP Knockin MDCKII Cells
1 Vial 2-6 Million Cells

Catalog Number **MTOX1304**

Store at –130 °C or below in liquid nitrogen vapor phase

TECHNICAL BULLETIN

Product Description

MDCK II - Madin-Darby canine kidney- is a subclone derived from the heterogenous parent line - MDCK (ECACC catalogue no. 85011435) MDCK II cells, which predominate in later passages from MDCK, are reported to display electrical resistance of 100 ohm/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]

Components

This product contains 1 cryovial of Canine MDR1 Knockout, Human BCRP Knockin MDCKII Cells which have been frozen at a density of 2-6 million cells. The vial contains sufficient cells to seed one 24 or 96 well Millicell® plate.

Cell Line Description

Parental Cell Line: ECACC Cat. 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

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.

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, it should be stored in liquid nitrogen vapor phase and not at –70 °C. Storage at –70 °C will result in loss of viability.

Precaution: It is recommended that protective gloves and clothing always be used, and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed 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.

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 Number PSRP010R5
- Millicell® 24-well receiver tray**,** Catalog Number SPMW010R
- Millicell® 96-well plate assembly, Catalog Number *PSRP004*
- Millicell® 96-well receiver tray, Catalog Number SPMW004R

MDCKII Medium Preparation

Prepare 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). This medium is formulated for use with a 5% CO_2 in air atmosphere.

Protocol for Thawing and Seeding Millicell® Plates

1. Pre-warm MDCKII Medium in 37 °C water bath.
2. Pipette 10 mL of pre-warmed, medium into a sterile centrifuge tube.
3. Prepare 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 °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 70% ethyl alcohol absorbent paper and place it under laminar flow hood.
8. Aseptically transfer the cell suspension to the centrifuge tube with pre-warmed medium and pellet cells by centrifugation at 800 RPM for three minutes.
9. Aspirate the media from the tube, and re-suspend the cell pellet in the appropriate amount of pre-warmed medium (13 mL for 24 well plates or 11 mL for 96 well plates.
10. Mix cells thoroughly and aliquot 500 µL per well (Millicell® 24-well plate assembly, Catalog Number PSRP010R5) or 100 µL per well (Millicell® 96-well plate assembly, Catalog Number *PSRP004*).
11. Add 25 ml media (Millicell® 24-well receiver tray**,** Catalog Number SPMW010R) or 5 ml media (Millicell® 96-well receiver tray, Catalog Number SPMW004R) to the bottom receiver tray of plate assembly.
12. Place the plate assembly in a 37 °C, 5% CO_2 incubator. Incubate the plate assembly until the cells form a confluent monolayer, replacing the medium in both the wells and receiver tray every 3-4 days. Assays may be performed post-confluent cells from days 2-5.

References

1. Bassett, A. R., et al., Highly Efficient Targeted Mutagenesis of Drosophila with the CRISPR/Cas9 System. Cell Rep. 2013: S2211-1247(13)00312-4.
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 (2010White 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 Absorptoin 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.

License Agreement – ADME/Tox Cell Lines, CRISPR Modified

This Product and its use are the subject of one or more of the following patents controlled by The Broad Institute, Inc. (BROAD), the Massachusetts Institute of Technology (MIT), or the President and Fellows of Harvard College (HARVARD): U.S. Patent Nos. 8,697,357; 8,771,945, and any substitutions, divisions, continuations, reissues, renewals, re-examinations or extensions, and corresponding foreign patent applications and patents.

BEFORE OPENING OR USING THIS PRODUCT, PLEASE READ THE TERMS AND CONDITIONS SET FORTH IN THIS LICENSE AGREEMENT. YOUR USE OF THIS PRODUCT SHALL CONSTITUTE ACKNOWLEDGMENT AND ACCEPTANCE OF THESE TERMS AND CONDITIONS. If you do not agree to use this Product pursuant to the terms and conditions set out in this License Agreement, please contact Sigma Technical Services within ten days of receipt to return the unused and unopened Product for a full refund; provided, however, that custom made Products may not be returned for a refund.

The purchase of this Product conveys to you, the buyer, the non-transferable right to use the purchased Product for Licensed Research Use (see definition below) subject to the conditions set out in this License Agreement. If you wish to use this Product for any purpose other than Licensed Research Use, you must first obtain an appropriate license (see information set out below).

This Product may not be used for any purpose other than Licensed Research Use. Product, as used herein, means the Cell Line and any derivative cells or cell lines created by the buyer which contain and/or incorporate genetic information derived from the Cell Line. Your right to use this Product for Licensed Research Use is subject to the following conditions and restrictions:

The type of license you have subject to this Agreement (Evaluation, Annual, Extended and Consumable) is listed on the outside of the package and/or on the invoice you received from us.

1. “Licensed Research Use” means any use for research purposes, other than:

a. Licensing, selling, distributing, or otherwise providing the product or modified versions of it to any third party other than Sigma and its affiliates as provided herein: provided however, that you may provide the product or modified versions of it to researchers within your research organization located at the same research facility or campus who are similarly bound to the use restrictions herein;

b. GMP production of therapeutic, diagnostic, prophylactic or other medicinal products intended for use in humans or non-human animals, or any other industrial use solely to the extent involving commercial sale of a product or service. If a molecule or any derivative of such molecule is used in or administered to humans, then the production of such molecule shall be deemed to be GMP production and therefore in violation of this License Agreement;

2. You may not transfer the Product, its components, or any materials made through the use of this Product, including further modified cells, to any third party without prior written approval of Sigma. Notwithstanding the foregoing, the Product or materials made through use of the Product may be transferred by you without such prior written approval to your legal affiliates or bona fide third party contractors performing paid work on your behalf, provided the use by such third party contractors is limited to performance of work for you and any results or product of such work shall not be shared by the third party contractor with any other person.

3. For Products covered by an “Evaluation” License, this Agreement shall expire ninety (90) days following your receipt of the Product.

4. For Products covered by an “Annual” License, this Agreement shall expire three hundred and sixty-five (365) days following your receipt of the Product.

5. For Products covered by an “Extended” License, this Agreement shall remain in force in perpetuity following your receipt of the Product.

6. For Products covered by a “Consumable” License, you may:

a. Not expand, propagate, seed, bank, cryopreserve or store the product, its components, or any materials made through the use of this Product, outside of the original shipping device, including but not limited to vial, transwell plate and Petaka cell culture device. The Product can only be transferred and/or incorporated in an end-use assay. All Products that are not utilized in an end-use assay shall be immediately destroyed.

b. Use Products for the provision of commercial services to a third party for monetary gain, but limited to only delivery of research results and data from use of the Product, and not the Product, its components, or any materials made through the use of this Product, including further modified cells.

7. Products delivered in a Petaka cell culture device can only be incorporated in an end-use assay for up to two (2) transwell plates (including, but not limited to 24-well or 96-well plates). All Products that are not utilized in an end-use assay for up to two (2) transwell plates shall be immediately destroyed.

8. BROAD, MIT and Harvard make no representations or warranties of any kind concerning the patent rights and hereby disclaim all representations and warranties, express or implied, including without limitation warranties of merchantability, fitness for a particular purpose, noninfringement of intellectual property rights of BROAD, MIT, Harvard or third parties, validity, enforceability and scope of patent rights, validity of any claims, whether issued or pending, and the absence of latent or other defects, whether or not discoverable. in no event shall BROAD, MIT, Harvard or their directors, trustees, officers, employees, agents, faculty, affiliated investigators or students, be liable for incidental or consequential damages of any kind, including without limitation economic damages or injury to property and lost profits, regardless of whether BROAD, MIT OR Harvard shall be advised, shall have other reason to know, or in fact shall know of the possibility of the foregoing.

9. Your right to use the Product will terminate immediately upon expiration of this License Agreement, or in the event that you fail to comply with these terms and conditions. You shall, upon such termination of your rights, destroy all Product, any modified versions of the Product, and components thereof in your control, and notify Sigma of such in writing.

Millicell is a registered trademark of Merck KGaA, Darmstadt, Germany

AA,PHC 02/17-1