Product Information Sheet for NR-2628

MDCK, Kidney (Canine), Working Cell Bank

Catalog No. NR-2628

(Derived from ATCC® CCL-34™)

For research use only. Not for human use.

Contributor and Manufacturer:

ATCC®

Product Description:

Madin-Darby canine kidney (MDCK)[1–5] cells are an adherent epithelial cell line derived from the kidney of an adult cocker spaniel in 1958. This cell line was deposited at ATCC® by S. H. Madin and N. B. Darby, Jr. The Working Cell Bank (WCB) is intended to be used as routine working inventory for this cell type.

Material Provided:

Each vial contains approximately 1 mL of cell culture suspension frozen in cell growth medium (95%) and DMSO (5%) cryo-preservatant. Sufficient cells are provided to initiate at least one new culture. The cell count, expressed as cells per vial, is shown on individual Certificates of Analysis for each lot.

Packaging/Storage:

This product was packaged aseptically in screw-capped plastic cryovials. It should be stored at cryogenic temperature (-100°C or colder), preferably in the vapor phase of a liquid nitrogen freezer. Storage at -70°C will result in loss of viability. To ensure the highest level of viability, the vial should be thawed and the culture initiated as soon as possible upon receipt. Any warming of the product during shipping and transfer must be avoided, as this will adversely affect the viability of the product after thawing. For transfer between freezers and shipping, MDCK cells may be placed on dry ice for brief periods, although use of a portable liquid nitrogen carrier is preferred. Please read the following recommendations prior to reconstituting this material.

Safety Precautions:

When handling frozen vials it is highly recommended that protective gloves, lab coat and full face mask be worn. Even brief exposure to the ultra-cold temperature can cause tissue damage from frostbite. Also, some vials may slowly fill with liquid nitrogen if they have been immersed during cryogenic storage. When thawing, the liquid nitrogen may rapidly expand as it changes to gas, breaking the vial or cap with explosive force, sending debris flying with enough velocity to cause injury. Store and use in areas with adequate ventilation.

Thawing and Growth:

Prior to thawing the MDCK WCB, prepare growth medium (GM) for use. MDCK cells are grown in Eagle's Minimum Essential Medium containing L-glutamine (HyClone SH30024.02) modified to contain 1X non-essential amino acids (NEAA, HyClone SH30238.01), 2 mM L-glutamine (HyClone SH30034.01), and 10% fetal bovine serum (FBS, HyClone SH30071.03IR).

Rapidly thaw the vial of MDCK cells in a 37°C water bath with gentle agitation. To reduce the risk of contamination, keep the cap and O-ring of the vial out of the water and repeatedly check the cap for tightness during thawing. Remove from the water bath immediately when thawed. Dry the vial with a sterile wiper, decontaminate using a wiper soaked with 70% isopropyl alcohol, and let the vial air dry. Aseptically open the vial, remove the vial contents and add to 14 mL of pre-warmed (37°C for 15 to 30 minutes) GM in a centrifuge tube. Centrifuge the cell suspension at approximately 275 X g for 10 minutes at 18–25°C. Discard the supernatant and resuspend the cell pellet in 15 mL of pre-warmed GM. Transfer the cell suspension into a 75 cm^2 tissue culture flask. Incubate the new culture at 37°C and 5% CO_2. Replace the GM with fresh GM every 2–3 days and incubate until the cell sheet is approximately 90–95% confluent.

Sub-culture procedure. Aseptically remove the GM and discard. Briefly rinse the cell layer with 4 to 15 mL of Ca^{2+}- and Mg^{2+}-free phosphate buffered saline (PBS, HyClone SH30028.02) to remove all traces of serum. Discard the PBS. Add 2 to 8 mL of trypsin (0.25%) with 0.2 g/L EDTA (HyClone SH30042.01) to the culture flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). *Note: To avoid clumping, do not agitate the cells by hitting or shaking the flask. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.* Add 8.0 mL of complete growth medium and aspirate cells by gently pipetting. Perform a cell count and add appropriate aliquots of the cell suspension to new culture vessels at a sub-cultivation ratio of 1:3 to 1:6. Adjust the volume of GM to 15–20 mL for a 75 cm^2 flask. Incubate cultures at 37°C and 5% CO_2. Replace the GM with fresh GM every 2–3 days and incubate until the cell sheet is approximately 90–95% confluent.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: MDCK, Kidney (Canine), Working Cell Bank, NR-2628."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in

Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2007; see www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.

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References:

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6. Caputo, J. L. "Biosafety Procedures in Cell Culture." J. Tissue Culture Methods 11 (1988): 223–227.
7. Fleming, D. O., J. H. Richardson, J. I. Tulis, and D. Vesley, eds. Laboratory Safety: Principles and Practice. 2nd ed. Washington, DC: ASM Press, 1995.

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