Product Information Sheet for NR-59612

Suspension Madin-Darby Canine Kidney (sMDCK) Research Cell Bank

Catalog No. NR-59612

(Derived from ATCC® CCL-34™)

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

FUJIFILM Diosynth Biotechnologies, College Station, Texas, USA

Product Description:

Suspension Madin-Darby Canine Kidney (sMDCK) research cell bank is a continuous cell line derived from ATCC® CCL-34™, an adherent cell line developed in 1958 from the normal kidney tissue of an adult female cocker spaniel, adapted to suspension culture under conditions free from animal-derived compounds, including bovine and porcine products at the Duke Human Vaccine Institute.[1,2] The sMDCK research cell bank is a model cell line widely used in biological research, including drug product manufacturing, biomedical research and toxicology. The complete production history can be found in Appendix I.

Intended Use

This product is intended for non-GMP laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

Material Provided:

Each vial contains approximately 1 mL of cell culture suspension frozen in cryopreservative (CryoStor® CS10, BioLife Solutions®). Sufficient cells are provided to initiate at least one new culture. The cell count, expressed as cells/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 -100°C or colder, preferably in the vapor phase of a liquid nitrogen freezer.

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, the 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:

It is highly recommended that appropriate personal protective equipment is always used when handling frozen vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris.

Growth Conditions:

Media:

MDXK Medium (Sartorius Xell GmbH 1010-0001) supplemented with 7.7 mM L-glutamine (Appendix II) or equivalent

Incubation:

Temperature: 37°C

Atmosphere: Aerobic with 5% CO_2 (humidified)

Shake Speed (rpm): 100

Propagation:

1. Prior to thawing cells, prepare the complete MDXK Medium according to Appendix II.
2. Warm the complete MDXK Medium protected from light at 37°C for 30 to 60 minutes.
3. Place the frozen vial in a 35°C to 37°C water bath and thaw for approximately 2 to 3 minutes. Immerse the vial just enough to cover the frozen material. Do not agitate the vial. Do not leave the vial in the water bath after it is thawed. 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.
4. Aseptically transfer 0.5 mL of pre-warmed complete MDXK Medium to the cryovial.
5. Gently transfer entire cell volume (approximately 1.5 mL) to a 50 mL conical tube containing 15 mL of complete MDXK Medium.
6. To ensure the majority of cells are collected, rinse the cryovial with 0.5 mL of complete MDXK Medium and transfer to the 50 mL conical tube.
7. Centrifuge the cell suspension at 330 × g at room temperature for 5 mins.
8. Discard the supernatant and resuspend the cell pellet in 30 mL of pre-warmed complete MDXK Medium.
9. Perform a cell count and determine viability.
10. Transfer the entire cell suspension to a sterile 125 mL Erlenmeyer shake flask.
11. Incubate the culture at 37°C in an aerobic atmosphere with 5% CO_2 on a cell culture shaker set to 100 rpm.
12. Observe the culture daily for approximately 3 to 4 days until culture reaches desired density.

Subculture Procedure:

1. When the culture is at or near peak density after approximately 3 to 4 days, perform a cell count and determine viability.

2. Determine a subculture schedule. For a 3-day schedule, use a seeding density of 8×10^6 cells per flask (approximately 2.67×10^5 cells/mL). For a 4-day schedule, use a seeding density of 8×10^6 cells per flask (approximately 2.67×10^5 cells/mL).
3. Transfer the appropriate seeding volume to a new sterile 125 mL Erlenmeyer shake flask. Incubate the culture at 37°C in an aerobic atmosphere with 5% CO_2 on a cell culture shaker set to 100 rpm.
4. On day 3 or 4, determine cell number and culture viability.
5. Repeat steps 3 and 4 above.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Suspension Madin-Darby Canine Kidney (sMDCK) Research Cell Bank, NR-59612."

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 (BMBL). 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

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References:

1. Balakumaran, B., Personal Communication.
2. Gaush, C. R., W. L. Hard and T. F. Smith. "Characterization of an Established Line of Canine Kidney Cells (MDCK)." Proc. Soc. Exp. Biol. Med. 122 (1966): 931-935. PubMed: 5918973.

Support Provided by NIAID

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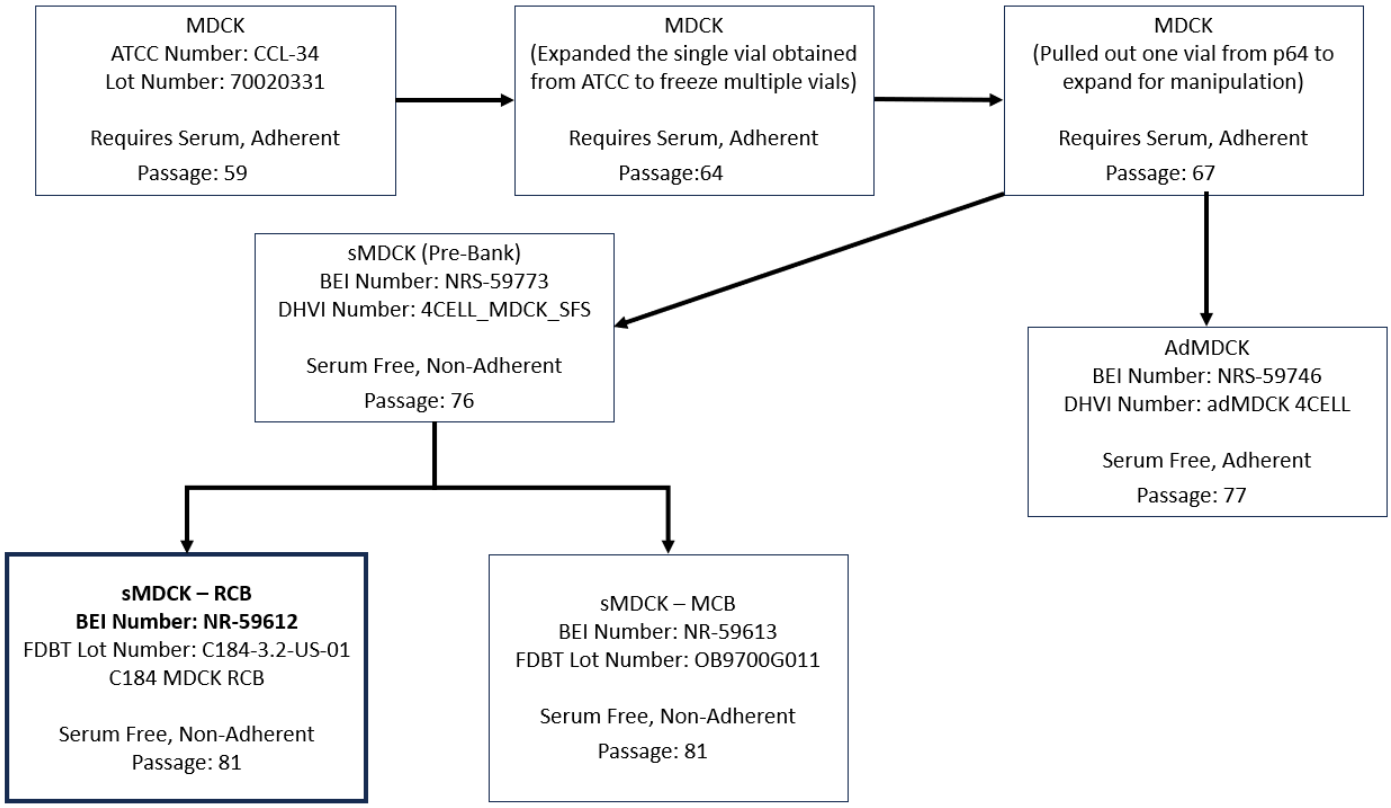
APPENDIX I: PRODUCTION OF sMDCK Research Cell Bank



APPENDIX II: MDXK Growth medium

1. Place a 200 mM L-Glutamine stock solution at 2°C to 4°C in a light-restricted container until thawed.
2. Aseptically add 40 mL of the 200 mM L-glutamine stock solution to a 1 L bottle of MDXK Medium.
3. Store the prepared medium at 2°C to 8°C covered with foil or otherwise protected from light.

Complete MDXK Medium:

MDXK Medium (Sartorius Xell GmbH 1010-0001)	1 L
L-Glutamine	7.7 mM

Note: Concentrations of L-glutamine up to 12 mM are acceptable and will not have a negative impact on cell growth.

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