Humanized Canine Kidney (hCK) Serum Free Suspension Research Cell Bank (non-GMP)

Catalog No. NR-59896

For research use only. Not for use in humans.

Contributor:
Biodefense Vaccines and Other Biological Products Development Section, NIH, NIAID, Rockville, Maryland, USA

Manufacturer:
IDT-Biologika, Rockville, Maryland, USA

Product Description:
NR-59896 is an engineered Madin-Darby canine kidney (MDCK) humanized cell line (hCK) that is more suitable for human influenza virus isolation and propagation. It was developed by Takada et al. by altering the expression levels of α-2,6-sialoglycans and α-2,3-sialoglycans of the host cell.[1,2]

IDT Biologika then adapted the hCK cell line for serum-free and suspension conditions, under contract.[1]

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 92.5% cryopreservation media [serum-free conditioned medium (46.25%), fresh serum-free medium (46.25%), supplemented with 7.5% dimethyl sulfoxide (DMSO)]. Sufficient cells are provided to initiate at least one new culture. The cell count, expressed as viable cells/vial, is shown on individual certificates of analysis for each lot.

Packaging/Storage:
This product was packaged aseptically in 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 the transfer between freezers and shipping, the cells may be placed on dry ice for brief periods, although the 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:
SFM4BHK21 Medium (Hyclone-Cytiva RR14443.01) supplemented with 1% glutamine.
Incubation:
Temperature: 37°C
Atmosphere: 5% CO_2 (humidified)
Shake Speed (rpm): 140
Propagation:
1. Prior to thawing cells, pre-warm SFM4BHK21 Medium protected from light at 37°C for 30 to 60 minutes.
2. 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.
3. Aseptically transfer 0.5 mL of pre-warmed SFM4BHK21 medium to the cryovial.
4. Gently transfer entire cell volume (approximately 1.5 mL) to a 50 mL conical tube containing 15 mL of pre-warmed SFM4BHK21 medium.
5. To ensure the majority of cells are collected, rinse the cryovial with 0.5 mL of complete SFM4BHK21 medium and transfer to the 50 mL conical tube.
6. Centrifuge the cell suspension at 330 × g at room temperature for 5 minutes.
7. Discard the supernatant and resuspend the cell pellet in 30 mL of pre-warmed complete SFM4BHK21 medium.
8. Perform a cell count and determine viability.
9. Transfer the entire cell suspension to a sterile 125 mL Erlenmeyer shake flask.
10. Incubate the culture at 37°C with 5% CO_2 on a cell culture shaker set to 100 rpm.
11. 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. Use a seeding density of 3 to 5 × 10^5 cells per flask.
3. Transfer the appropriate seeding volume to a new sterile 125 mL Erlenmeyer shake flask. Incubate the culture at 37°C with 5% CO_2 on a cell culture shaker set to 100 rpm.
4. On day of harvest, determine cell number and culture viability.
5. Repeat steps 3 and 4 above.

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Citation:
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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). Current Edition. Washington, DC: U.S. Government Printing Office.

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

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Use Restrictions:
Commercial use of Humanized Canine Kidney (hCK) Serum Free Suspension Research Cell Bank (non-GMP), NR-59896, requires a license agreement with WARF, including under issued US patent (11,851,648) and pending or issued patents in the US and other countries. Please contact WARF at licensing@warf.org for further information.

References:
1. NIH/NIAID, Personal Communication.
2. Takada, K., et al. "A Humanized MDCK Cell Line for the Efficient Isolation and Propagation of Human Influenza Viruses." Nat. Microbiol. 4 (2019): 1268-1273. PubMed: 31036910.

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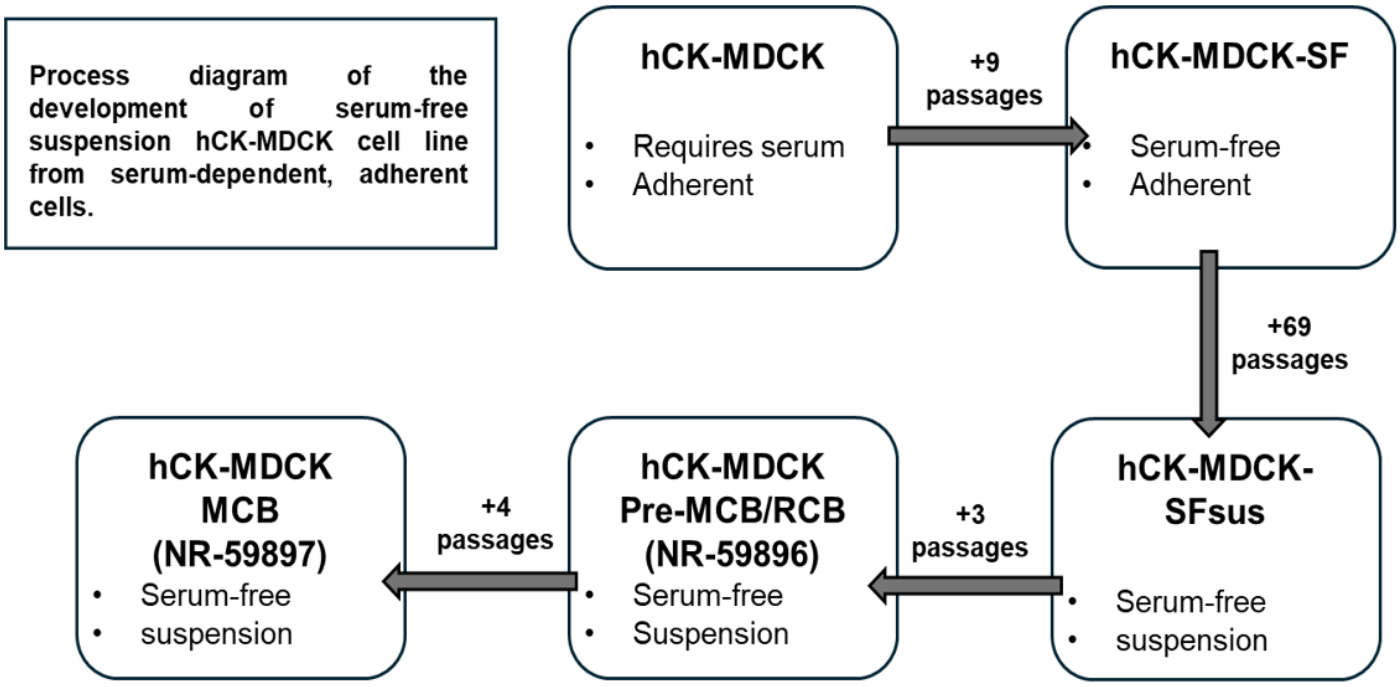
APPENDIX I: PRODUCTION OF hCK-MDCK RESEARCH CELL BANK



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