Product Information Sheet for NR-49169

R05T, *Rousettus aegyptiacus* (Egyptian fruit bat) Immortalized Fetal Cell Line

Catalog No. NR-49169

For research use only. Not for human use.

Contributor:
Ingo Jordan, Ph.D., Director, Viral Vaccine Development, ProBioGen, AG, Berlin, Germany

Manufacturer:
BEI Resources

Product Description:
The R05T cell line was generated from cells isolated from the head of an Egyptian fruit bat (*Rousettus aegyptiacus*) fetus. Cultured primary cells were immortalized by transfection with an adenovirus 5 E1A/E1B expression plasmid.[1] *R. aegyptiacus* has been identified as a natural reservoir for marburgviruses.[2,3] However, experimental inoculation studies indicated very limited susceptibility to ebolavirus infection, suggesting that this species is an unlikely source for ebolaviruses in nature.[4]

NR-49169 deposited at BEI Resources was confirmed to be of bat origin and remained free of detectable cross-contamination with cells of 12 other mammalian species, even after ten passages of the original seed cells received from the contributor.

Material Provided:
Each vial contains approximately 1 mL of cell culture suspension frozen in DMEM:F12 medium (65%), fetal bovine serum (25%), and DMSO (10%) cryopreservative. 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 product 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 insure 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:
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 R05T cells, prepare growth medium (GM) for use. R05T cells are grown in DMEM:F12 medium (ATCC 30-2006 or equivalent) supplemented with 5% fetal bovine serum (FBS; ATCC 30-2020 or equivalent). This GM is formulated for use with a 5% CO_2 in air atmosphere.

Rapidly thaw the vial of R05T 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 4 mL of pre-warmed (37°C for 15 to 30 minutes) GM in a centrifuge tube. Centrifuge the cell suspension at 125 to 200 × g for 8 to 10 minutes at 18 to 25°C. Discard the supernatant and resuspend the cell pellet in 10 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. After the cells have attached, replace the GM with fresh GM every 2 to 3 days. The cells should form a confluent monolayer within 7 days, and should be sub-cultured every 3 to 7 days thereafter.

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 Dulbecco's phosphate-buffered saline (PBS; ATCC 30-2200) to remove all traces of serum. Discard the PBS. Add 2 to 8 mL of 0.25% trypsin-EDTA (ATCC 30-2101 or equivalent) to the culture flask and incubate the flask at 37°C until cell layer is dispersed (usually within 3 minutes but no longer than 15 minutes). *Note: To avoid clumping, do not agitate the cells by hitting or shaking the flask.* Add an equal volume of GM 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 1:10. Adjust the volume of GM to 15 to 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 to 3 days.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH:

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R05T, *Rousettus aegyptiacus* (Egyptian fruit bat), Immortalized Fetal Cell Line, NR-49169."

Biosafety Level: 2

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, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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NR-49169 is claimed in U.S. Patent Number 8,679,839, and the continuations, continuations-in-part, re-issues and foreign counterparts thereof. Commercial sale of any products or services based on this material will require a license from ProBioGen, AG, Berlin, Germany.

References:

1. Jordan, I., et al. "Cell Lines from the Egyptian Fruit Bat are Permissive for Modified Vaccinia Ankara." Virus Res. 145 (2009): 54-62. PubMed: 19540275.
2. Towner, J. S., et al. "Isolation of Genetically Diverse Marburg Viruses from Egyptian Fruit Bats." PLoS Pathog. 5 (2009): e1000536. PubMed: 19649327.
3. Amman, B. R., et al. "Seasonal Pulses of Marburg Virus Circulation in Juvenile *Rousettus aegyptiacus* Bats Coincide with Periods of Increased Risk of Human Infection." PLoS Pathog. 8 (2012): e1002877. PubMed: 23055920.
4. Jones, M. E., et al. "Experimental Inoculation of Egyptian Rousette Bats (*Rousettus aegyptiacus*) with Viruses of the Ebolavirus and Marburgvirus Genera." Viruses 7 (2015): 3420-3442. PubMed: 26120867.

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