Cercopithecus aethiops Kidney Epithelial Cells with High Expression of Human Furin (Vero-Furin)

Catalog No. NR-55312

For research use only. Not for use in humans.

Contributor:
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Manufacturer:
BEI Resources

Product Description:
NR-55312 contains a preparation of African green monkey *(Cercopithecus aethiops)* kidney epithelial cells with high expression of human furin (Vero-furin).[1] A clone was constructed by standard molecular cloning techniques into a bicistronic human furin-expressing pcDNA3.1 vector (pFIRB), and the vector was stably transfected into Vero cells, resulting in Vero-furin.[2] These cells are useful for the production of viruses with more effective furin cleavage.[2]

Material Provided:
Each vial contains approximately 1 mL of cell culture suspension frozen in cell growth media (90%) 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 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. Storage at -70°C will result in loss of viability. To ensure the highest level of viability, the vial should be quick-thawed at 37°C 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 Vero-furin cells, prepare growth medium (GM) for use. Vero-furin cells are grown in Dulbecco's Modified Eagle's Medium containing 2 mM L-glutamine, supplemented with 10% fetal bovine serum (ATCC® 30-2020™). This GM is formulated for use with a 5% CO_2 in air atmosphere. Note: Cells should be suspended in 90% Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum, and 10% DMSO cryopreservative if being frozen in aliquots for future culture.

Rapidly thaw the vial of 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 inside a biosafety cabinet. Aseptically open the vial, remove the vial contents and add to 4 mL of GM in a centrifuge tube. Centrifuge the cell suspension at 125 to 200 × g for 8 to 10 minutes at 18°C 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. Replace the GM with fresh GM every 2 to 3 days and incubate until the cell sheet is approximately 80% 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 Dulbecco's phosphate-buffered saline (PBS) to remove all traces of serum. Discard the PBS. Add 2 to 8 mL of 0.25% trypsin-EDTA 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.* Following dissociation, dilute the cell suspension with an equal volume of GM. Centrifuge the cell suspension at 125 × g for 8 to 10 minutes at 18°C to 25°C. Discard the supernatant and resuspend the cell pellet in 10 mL of pre-warmed GM. 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 1:3 to 1:4. 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 and incubate until the cell sheet is approximately 80% confluent.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Cercopithecus aethiops* Kidney Epithelial Cells with High Expression of Human Furin (Vero-Furin), NR-55312."

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. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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References:
1. Pierson, T. C., Personal Communication.
2. Mukherjee, S., et al. “Enhancing Dengue Virus Maturation Using a Stable Furin Over-Expressing Cell Line.” Virology 497 (2016): 33-40. PubMed: 27420797.

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