Product Information Sheet for NR-53726

African Green Monkey Kidney Epithelial Cells (Vero E6) Expressing High Endogenous Angiotensin-Converting Enzyme 2

Catalog No. NR-53726

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

NR-53726 consists of a preparation of African green monkey *(Cercopithecus aethiops)* kidney epithelial cells (Vero E6) expressing high endogenous angiotensin-converting enzyme 2 (ACE2). This cell line was obtained by sorting Vero E6 cells for cells expressing high levels of endogenous ACE2.[1]

ACE2 is an enzyme established as the functional receptor by which SARS-Related Coronavirus 2 (SARS-CoV-2) enters host target cells.[2] The SARS-CoV-2 spike glycoprotein mediates viral binding to the host ACE2 receptor.[3] This protein forms a trimer, and when bound to ACE2, allows fusion of the viral and cellular membranes, allowing viral entry and replication. The interaction of ACE2 and the spike glycoprotein during viral infection is currently under study.[2,3]

Material Provided:

Each vial contains approximately 1 mL of cell culture suspension frozen in cell growth medium (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:

NR-53726 was packaged aseptically in screw-capped plastic cryovials. The product is provided frozen and 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 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 cells, prepare growth medium (GM) for use, consisting of Dulbecco's Modified Eagle's Medium containing 4 mM L-glutamine, 4500 mg/L glucose, 1 mM sodium pyruvate and 1500 mg/L sodium bicarbonate supplemented with 10% irradiated fetal bovine serum (Corning® 35-070-CV). This GM is formulated for use with a 5% CO_2 in air atmosphere.

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. 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 × *g* for 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. 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). 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 1:5 to 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 and incubate until the cell sheet is approximately 90% confluent.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: African Green Monkey Kidney Epithelial Cells (Vero E6) Expressing High Endogenous Angiotensin-Converting Enzyme 2, NR-53726."

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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. 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. Baric, R., Personal Communication.
2. Kai, H. and M. Kai. "Interactions of Coronaviruses with ACE2, Angiotensin II, and RAS Inhibitors - Lessons from Available Evidence and Insights into COVID-19." Hypertens. Res. 43 (2020): 648-654. PubMed: 32341442.
3. Hulswit, R. J. G., C. A. M. de Haan and B.-J. Bosch. "Coronavirus Spike Protein and Tropism Changes." Adv. Virus Res. 96 (2016): 29-57. PubMed: 27712627.

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