Product Information Sheet for NR-55340

Homo sapiens Lung Adenocarcinoma Cells (Clone 2B4) with High Expression of Human Angiotensin-Converting Enzyme 2 [Calu-3 (Clone 2B4)-ACE2]

Catalog No. NR-55340

For research use only. Not for use in humans.

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Manufacturer:
BEI Resources

Product Description:
NR-55340 [Calu-3 (Clone 2B4)-ACE2] contains a preparation of *Homo sapiens* lung adenocarcinoma cells (Calu-3) which were cloned by standard limiting dilution to select a clone that expresses increased levels of angiotensin-converting enzyme 2 (ACE2).[1,2] ACE2 is a human receptor that is expressed widely, including in heart, kidney, small intestine and lung cells, and is involved in the regulation of hypertension and other cardiovascular diseases.[3] The SARS-Related Coronavirus 2 (SARS-CoV-2) spike glycoprotein mediates viral binding to the host ACE2 receptor.[4]

Material Provided:
Each vial contains approximately 1 mL of cell culture suspension frozen in 70% Dulbecco's Modified Eagle's Medium containing 20% fetal bovine serum and 10% DMSO 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 Calu-3 (Clone 2B4)-ACE2 cells, prepare growth medium (GM) for use. Calu-3 (Clone 2B4)-ACE2 cells are grown in Dulbecco's Modified Eagle's Medium containing 4 mM L-glutamine, 4500 milligrams per liter glucose, 1 mM sodium pyruvate and 1500 milligrams per liter sodium bicarbonate, supplemented with 20% 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 70% Dulbecco's Modified Eagle's Medium containing 20% 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. 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. This may take up to 10 days.

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. This may take up to 10 days.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Homo sapiens* Lung Adenocarcinoma Cells (Clone 2B4) with

High Expression of Human Angiotensin-Converting Enzyme 2 [Calu-3 (Clone 2B4)-ACE2], NR-55340."

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. 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. Tseng, C.-T. K., Personal Communication.
2. Kumar, S., et al. "Clinically Relevant Cell Culture Models and their Significanse in Isolation, Pathogenesis, Vaccine Development, Repurposing and Screening of New Drugs for SARS-CoV-2: A Systematic Review." Tissue Cell 70 (2021): doi: 10.1016/jtice.2021.101497. PubMed: 33550034.
3. Yoshikawa, T., et al. "Dynamic Innate Immune Responses of Human Bronchial Epithelial Cells to Severe Acute Respiratory Syndrome-Associated Coronavirus Infection." PLoSOne 5 (2010): e8729. PubMed: 20090954.
4. 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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