

***Homo sapiens* Embryonic Kidney Epithelial Cells Expressing Transmembrane Protease, Serine 2 and Human Angiotensin-Converting Enzyme 2 [293T-ACE2.TMPRSS2 (mCherry)]**

Catalog No. NR-55293

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

NR-55293 [293T-ACE2.TMPRSS2 (mCherry)] contains a preparation of *Homo sapiens* embryonic kidney epithelial cells (HEK-293T) which were modified to express the transmembrane protease, serine 2 (TMPRSS2) and 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 TMPRSS2 has been shown to be involved in proteolytically activating SARS-CoV. Cells expressing human TMPRSS2 were found to be highly susceptible to infection by SARS-CoV-2.⁵

293T-ACE2.TMPRSS2 (mCherry) stable cell line was created by transducing 293T-ACE2 cells (BEI Resources NR-52511) with lentivirus encoding TMPRSS2 and mCherry as a bicistronic transcript.^{1,2,6} The bulk transduced population was single-cell sorted by flow cytometry based on mCherry positivity and the clone displaying intermediate levels of mCherry expression and maximum pseudovirus infectivity titer was expanded as 293T-ACE2.TMPRSS2 (mCherry) cells.²

Material Provided:

Each vial contains approximately 1 mL of cell culture suspension frozen in Dulbecco's Modified Eagle's Medium containing 10% heat-inactivated fetal bovine serum 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 293T-ACE2.TMPRSS2 (mCherry) cells, prepare growth medium (GM) for use. 293T-ACE2.TMPRSS2 (mCherry) cells are grown in Dulbecco's Modified Eagle's Medium containing 4 mM L-glutamine, 4500 mg per L glucose, 1 mM sodium pyruvate and 1500 mg per L sodium bicarbonate, supplemented with 10% heat-inactivated fetal bovine serum (ATCC® 30-2020). This GM is formulated for use with a 5% CO₂ 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 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 cm² tissue culture flask. Incubate the new culture at 37°C and 5% CO₂. 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 Ca²⁺- and Mg²⁺-free Dulbecco's phosphate-buffered saline (PBS) to remove all traces of serum. Discard the PBS. Add 2 to 8 mL of TrypLE (Gibco® 12563-029) 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. *Note: Culture medium will not neutralize TrypLE, so it must be washed off cells.* 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 cm² flask. Incubate cultures at 37°C and 5% CO₂. 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: *Homo sapiens* Embryonic Kidney Epithelial Cells Expressing Transmembrane Protease, Serine 2 and Human Angiotensin-Converting Enzyme 2 [293T-ACE2.TMPRSS2 (mCherry)], NR-55293.”

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. Weiss, C., Personal Communication.
2. Neerukonda, S. N., et al. “Establishment of a Well-Characterized SARS-CoV-2 Lentiviral Pseudovirus Neutralization Assay using 293T Cells with Stable Expression of ACE2 and TMPRSS2.” PLoS One 16 (2021): e0248348. PubMed: 33690649.
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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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