

## Vero-E6 Ebola Reporter Cell Line, Clone B8

### Catalog No. NR-53949

**For research use only. Not for use in humans.**

#### Contributor:

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#### Manufacturer:

BEI Resources

#### Product Description:

NR-53949 contains a preparation of *Cercopithecus aethiops* kidney epithelial cells (Vero E6; ATCC® CRL-1586™), which have been modified to contain a minigenome encoding a green fluorescent protein, zsGreen (ZsG), that is activated upon infection with Ebolavirus (EBOV).<sup>1,2</sup>

The minigenome consists of a codon-optimized zsG gene flanked by a 176 base pair Ebola minimal promoter region, followed by the untranslated region (UTR) of the L gene between the polyadenylation signal and the stop codon on the 5' end and the preceding sequence of the nucleotide open reading frame on the 3' end. The 3' end is trimmed by the "supercut" hepatitis delta ribozyme.<sup>2</sup> The negative sense transcript expression is driven by the constitutive cytomegalovirus (CMV) promoter and translation of the reporter gene occurs upon infection with EBOV. Transcription of the negative-sense strand is carried out by the host RNA polymerase II.<sup>2,3</sup>

The construct was initially created and optimized in a pCDNA5/FRT vector and subsequently transferred into the piggyBAC transposon vector PB\_CMV\_MCS\_E81-Puro. Stable lines were generated by transfection of the resulting piggyBAC construct together with the Super piggy-BAC transposase construct. Clones were selected and expanded under 30 micrograms per milliliter puromycin, and the best-performing clones were expanded further, with the selection of one such line resulting in NR-53949.<sup>2</sup>

#### Material Provided:

Each vial contains approximately 1.0 mL of cell culture suspension frozen in Dulbecco's Modified Eagle's Medium containing 10% 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 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 Vero-E6 Ebola Reporter cells, prepare growth medium (GM) for use. Vero-E6 Ebola Reporter 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 10% fetal bovine serum (ATCC® 30-2020™). After the initial passage, 10 micrograms per milliliter puromycin should be added to the GM. This GM is formulated for use with a 5% CO<sub>2</sub> 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 cm<sup>2</sup> tissue culture flask. Incubate the new culture at 37°C and 5% CO<sub>2</sub>. Replace the GM with fresh GM containing 10 micrograms per milliliter puromycin 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<sup>2+</sup>- and Mg<sup>2+</sup>-free Dulbecco's phosphate-buffered saline (PBS) to remove all traces of serum. Discard the PBS. Add 2 to 8 mL of 0.05% trypsin-EDTA to the culture flask and incubate the flask at 37°C until the 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 1:3 to 1:4. Adjust the volume of GM containing 10 micrograms per milliliter puromycin to 15 to 20 mL for a 75 cm<sup>2</sup>

flask. Incubate cultures at 37°C and 5% CO<sub>2</sub>. Replace the GM containing 10 micrograms per milliliter puromycin with fresh GM containing 10 micrograms per milliliter puromycin 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: Vero-E6 Ebola Reporter Cell Line, Clone B8, NR-53949.”

**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](http://www.cdc.gov/biosafety/publications/bmbl5/index.htm).

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Hera Bio Labs. Please contact [jlesnik@systembio.com](mailto:jlesnik@systembio.com) for a copy of the Limited Use Label License (LULL) and for any questions regarding the LULL.

**References:**

1. Kainulainen, M., Personal Communication.
2. Kainulainen, M., et al. “Rapid Determination of Ebolavirus Infectivity in Clinical Samples Using a Novel Reporter Cell Line.” J. Infect. Dis. 216 (2017): 1380-1385. PubMed: 29029133.
3. Schuit, M. et al. “The Use of an Ebola Virus Reporter Cell Line in a Semi-Automated Microtitration Assay.” J. Virol. Methods 292 (2021): 114116. PubMed: 33689788.

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