Vero, Kidney (African green monkey), Expressing Luciferase (Luc2p)

Catalog No. NR-10385

For research use only. Not for human use.

Contributor:

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Product Description:

Vero cells are an adherent epithelial cell line derived from the kidney of an African green monkey (*Cercopithecus aethiops*). NR-10385 consists of Vero cells engineered to stably express the Luc2p variant of luciferase.[1] Luc2p is tagged with a proline- glutamic acid-serine-threonine (PEST) sequence, which promotes rapid turnover by targeting the protein to the proteosome for degradation. Since measurable luciferase activity is due to newly synthesized protein, luciferase decreases when protein synthesis is inhibited. Vero cells expressing Luc2p have been used in the development of a quantitative and sensitive luciferase-based assay for bacterial toxins that inhibit protein synthesis.[2]

Material Provided:

Each vial contains approximately 1 mL of cell culture suspension frozen in cell growth medium (90%) and DMSO (10%) cryo-preservatant. Sufficient cells are provided to initiate at least one new culture. The viable cell count, expressed as viable cells per vial, is shown on individual Certificates of Analysis for each lot. Note: These cells are not recommended for use beyond passage 22 as they begin to lose sensitivity.

Packaging/Storage:

This product was packaged aseptically in screw-capped plastic cryovials. It should be stored at cryogenic temperature (-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 media (GM) for use. Cells are grown in Minimal Essential Medium containing Earle's salts and non-essential amino acids (MEM; Invitrogen™ 10370) modified to contain 1 mM sodium pyruvate (Invitrogen™ 11360), 2 mM L-glutamine (Invitrogen™ 25030-081) and 10% fetal bovine serum (ATCC® 30-2020). This media is formulated for use with a 5% CO_2 in air atmosphere.

Rapidly thaw a 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 air dry. Aseptically open the vial, remove the vial contents and add to 10 mL of pre-warmed (37°C for 15 to 30 minutes) GM in a centrifuge tube. Centrifuge the cell suspension at approximately 180 X g for 10 minutes. Discard the supernatant and resuspend the cell pellet in 10-15 mL of pre-warmed (37°C) 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 2-3 days and incubate until the cell sheet is approximately 80-95% confluent.

Sub-culture procedure. Aseptically remove the GM and discard. Briefly rinse the cell layer with 5 to 15 mL of Ca^{2+}- and Mg^{2+}-free phosphate buffered saline (PBS, Invitrogen™ 14190) to remove all traces of serum. Discard the PBS. Add 3 to 5 mL of trypsin (0.25%) with 0.38 g/L EDTA (Invitrogen™ 25200-056) to the flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). *Note: To avoid clumping, do not agitate the cells by hitting or shaking the flask. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.* Add 5.0 mL of complete growth medium 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 1:10. Adjust the volume of GM to 10-20 mL for a 75 cm^2 flask. Incubate cultures at 37°C and 5% CO_2. Replace the GM with fresh GM every 3-4 days and incubate until the cell sheet is approximately 80-95% confluent.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: Vero, Kidney (African green monkey), Expressing

Luciferase (Luc2p), NR-10385."

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. 5th ed. Washington, DC: U.S. Government Printing Office, 2007; see www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.

This cell line is not known to harbor an agent that causes disease in healthy adult humans. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. This cell line has **NOT** been screened for hepatitis B virus, human immunodeficiency virus or other adventitious agents. Cell lines derived from primate tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens. ATCC® recommends that appropriate safety procedures[3,4] are used when handling all cell lines, especially those derived from human or other primate material.

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References:

1. McGannon, C. M., C. A. Fuller, and A. A. Weiss. "Different Classes of Antibiotics Differentially Influence Shiga Toxin Production." Antimicrob. Agents Chemother. 2010 June 28 [Epub ahead of print]. PubMed: 20585113.
2. Zhao, L. and D. B. Haslam. "A Quantitative and Highly Sensitive Luciferase-Based Assay for Bacterial Toxins that Inhibit Protein Synthesis." J. Med. Microbiol. 54 (2005): 1023-1030. PubMed: 16192432.
3. Caputo, J. L. "Biosafety Procedures in Cell Culture." J. Tissue Culture Methods 11 (1988): 223-227.
4. Fleming, D. O., J. H. Richardson, J. I. Tulis, and D. Vesley, eds. Laboratory Safety: Principles and Practice. 2nd ed. Washington, DC: ASM Press, 1995.

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