

BHK-21 Cell Line Harboring SARS-CoV-2-Replicon Containing NanoLuc®-Neo Reporters and NSP1 Mutations (K164A/H165A)

Catalog No. NR-58876

This reagent is the tangible property of the U.S. Government.

For research use only. Not for use in humans.

Contributor:

Tony T. Wang, Principal Investigator, Division of Viral Products, Center for Biologics Evaluation and Research and Review (CBER), U.S. Food and Drug Administration (FDA), Silver Spring, Maryland, USA

Manufacturer:

BEI Resources

Product Description:

NR-58876 is a stable baby hamster kidney fibroblast (BHK-21) cell line harboring a self-replicating severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) replicon in which the spike (S) gene was replaced with a nanoluciferase reporter gene (NanoLuc®), and the envelope (E) and membrane (M) genes are replaced with neomycin phosphotransferase gene (*neo*). The non-structural protein 1 (*nsp1*) gene was mutated to introduce two point mutations resulting in K164A and H165A substitutions to reduce NSP1-induced cellular toxicity.¹

Material Provided:

Each vial contains approximately 1.0 mL of cell culture suspension frozen in Fetal Bovine Serum (FBS) (90%) and DMSO (10%) cryopreservative. Sufficient cells are provided to initiate at least one new culture. The cell count, expressed as cells/vial, is shown on individual Certificates of Analysis for each lot.

Packaging/Storage:

NR-58876 was packaged aseptically in screw-capped plastic cryovials. The product should be stored at -100°C or colder, preferably in the vapor phase of a liquid nitrogen freezer.

Note: Do not under any circumstances store vials at temperatures warmer than -100°C. Storage under these conditions will result in the death of the culture.

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.

Functional Activity:

Approximately 3×10^4 replicon cells per well were seeded in 48-well plates. After 24 hours, the cells were treated with 5 mM of three compounds or DMSO negative control. After 6 days, luciferase activity was measured. A decline in luciferase activity was noted for GC376, a protease inhibitor, Remdesivir, a nucleotide prodrug, and Nirmatrelvir, an oral protease inhibitor, relative to the DMSO negative control wells.

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:

Note: Extended incubation and sub-culturing may be required to produce sufficient cells for downstream applications.

Prior to thawing the modified BHK-21 cells, prepare growth medium (GM) for use. NR-58876 cells are grown in Dulbecco's Modified Eagle's Medium, supplemented with 10% fetal bovine serum (ATCC® 30-2020™) and 200 µg/mL G418. 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 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.** 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 up to 1:10. 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: BHK-21 Cell Line Harboring SARS-CoV-2-Replicon Containing NanoLuc®-Neo Reporters and NSP1 Mutations (K164A/H165A), NR-58876.”

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 \(BMBL\)](#). 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at www.beiresources.org.

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use, and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure the authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers, and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

Use Restrictions:

This material is distributed for internal research, and non-commercial purposes only. This material, its product, or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products, or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

NR-58876 is claimed in U.S. Provisional Patent Application number 63/275251 filed 03NOV2021, and the continuations,

continuations in part, re-issues and foreign counterparts thereof.

Material may not be used for commercial purposes without a license from the United States Food and Drug Administration (U.S. FDA). Contact Mr. William Ronnenberg (William.ronnenberg@fda.hhs.gov) for a commercial license.

Use of NR-58876 is subject to the terms of the Limited Use Label License for NanoLuc® Products. You may use this product for research use only; no commercial use is allowed. Commercial use means any and all uses of this product or derivatives by a party in exchange for consideration, including, but not limited to, (1) use in further product manufacture; (2) use in provision of services, information or data; and (3) resale of the product or its derivatives, whether or not such product or derivatives are resold for use in research. Users shall have no right to modify or otherwise create variations of the nucleotide sequence of the luciferase gene contained in NR-58876. No other use or transfer of this product or derivatives is authorized without the prior express written consent of Promega Corporation.

In addition, users of NR-58876 must (1) use Nano-Glo®-branded luminescent assay reagents (LARs) manufactured by Promega and sold by ATCC® within a complete assay kit or sold by Promega as stand-alone LARs for all determinations of luminescence activity of this product and its derivatives, or (1a) contact Promega to obtain a license for use of the luciferase gene contained in this product and its derivatives.

References:

1. Liu, S., et al. “Stable Cell Clones Harboring Self-Replicating SARS-CoV-2 RNAs for Drug Screen.” *J. Virol.* 96 (2022): e0221621. PubMed: 35080424.

ATCC® is a trademark of the American Type Culture Collection.

