

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

Catalog No. NR-58876

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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.

Lot: 70057413

Manufacturing Date: 09DEC2022

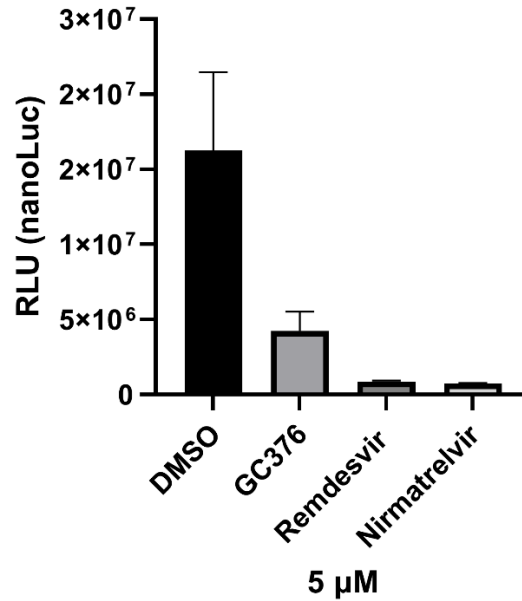
TEST	SPECIFICATIONS	RESULTS
Growth Properties	Adherent	Adherent
Morphology	Fibroblast	Fibroblast
Multiplex PCR Amplification of Cytochrome C Oxidase I (COI) Gene	Hamster (<i>Mesocricetus auratus</i>) origin No evidence of another species	Hamster (<i>Mesocricetus auratus</i>) origin No evidence of another species
Total Cell Count	Report results	3.6 × 10 ⁶ cells/vial
Post-Freeze Viability	≥ 50%	80.0%
Functionality of SARS-CoV-2 replicon^{1,2} GC376 (protease inhibitor) Remdesivir (nucleotide prodrug) Nirmatrelvir (oral protease inhibitor)	Reduction in NanoLuc® expression Reduction in NanoLuc® expression Reduction in NanoLuc® expression	Reduction observed (Figure 1) Reduction observed (Figure 1) Reduction observed (Figure 1)
Sterility (21-day incubation) Harpo's HTYE broth, 37°C and 26°C, aerobic ³ Trypticase Soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO ₂	No growth No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth
Mycoplasma Contamination Hoechst DNA stain Agar and broth culture (14-day incubation at 37°C) DNA detection by PCR of extracted Test Article nucleic acid	None detected None detected None detected	None detected None detected None detected

¹Testing was performed by the contributor on lot 70056891.

²Approximately 3 × 10⁴ replicon cells/well were seeded in a 48-well plate. Twenty-four hours later, cell culture media was replaced with media containing 5 μM of compounds or the same volume of diluent DMSO. After 6 days of treatment at 37°C, cells were assayed for NanoLuc® expression using Nano-Glo® Luciferase Assay System (Promega N1130).

³Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: Expression Levels NanoLuc® Activity



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