



Neutralization of SARS-CoV-2 Renilla luciferase Reporter Virus Particles using 293T-hsACE2 cells

Safety note: SARS-CoV-2 RVPs are created using a second-generation lentiviral system and should be used under BSL2 or enhanced BSL2 laboratory conditions. Use of SARS-CoV-2 RVPs requires infectious agent training. Always wear gloves, eye protection, and a lab coat when using RVPs. Filter tips on pipettors are recommended when handling infectious agents. Please see the SARS-CoV-2 RVP Safety Data Sheet for full safety information. <https://www.integralmolecular.com/sarscov2rvpddocuments/>

Required Reagents

- SARS-CoV-2 Luciferase Reporter Virus Particles (RVPs) (Integral Cat# RVP-7XXL)
- Clonal 293T-hsACE2 cells (Integral Cat# C-HA102)
- 96 or 384 well plate (white opaque bottom, tissue culture treated, or Poly-D-Lysine coated)
- Cell Culture media (DMEM + 10% FBS + 10 mM HEPES + 1x Penicillin-Streptomycin + 1% Sodium Pyruvate + 1% MEM NEAA + 1% L-Glutamine) (Corning Cat# 10017CM, Summerlin Cat# SS-100, Corning Cat# 25-060-CI, Corning Cat# 30-002-CI, Corning Cat# 25-000-CI, Corning Cat# 25-025-CI, Corning Cat# 25-015-CI)
- 0.05% Trypsin-EDTA (Corning Cat# 25-052-CI)
- Neutralizing antibody (see below) or serum (provided by the end user)
 - o Integral antibody IM-COV2-F06 for Wuhan-hu-1, Alpha, D614G, and select Omicron RVPs*
 - o Integral antibody IM-COV2-G06 for Wuhan-hu-1, Alpha, and Delta RVPs
- Renilla-Glo Luciferase Assay System (Promega #E2710)

*Omicron variants BA.1, BA.1.1, BA.2, and BA.2.75

NOTE: Catalog numbers correspond to our recommended products. They can be substituted as desired.

NOTE: The luciferase RVPs contain luciferase from sea pansy (*Renilla reniformis*), therefore a reagent compatible with Renilla luciferase is required. Detection reagents for firefly luciferase will not yield a positive result.

A. Preparation of serum or antibody dilutions

- Prepare dilutions of neutralizing serum or antibody at 2X the desired final concentration in Cell Culture media. Make sure to leave a negative control without antibody or serum - that will be your control for 100% infectivity.
- You will need a total volume of 10 μ L/well for a 384-well plate or 50 μ L/well for a 96-well plate, of your 2X diluted antibody/serum.

B. Incubating RVPs with diluted antibody or serum

- Remove an aliquot of frozen RVPs, place in a 37°C water bath for 3 minutes until just thawed, and then immediately place on ice (**see Note 1**).
- Gently mix the RVPs tube by inversion.
- Dilute the recommended amount of RVP per well in Cell Culture media for a total volume of 10 μ L/well for a 384-well plate or 50 μ L/well for a 96-well plate. The recommended amount of RVP



per well can be found in the Tech Data Sheet for each lot of RVP but should be determined empirically based on your needs.

- d. Mix the RVPs with the antibody/serum dilutions (1:1). This will be a total volume of 100 μ L/well for a 96-well plate or 20 μ L/well for a 384-well plate, with the serum/MAB now at a 1X concentration.
- e. Incubate at 37°C for 1 h.

C. Infecting 293T-hsACE2 cells with RVPs

- a. Lift a sub-confluent flask of 293T-hsACE2 cells using 0.05% Trypsin-EDTA, stop trypsinization by adding Cell Culture media (twice the volume of the Trypsin-EDTA added), pellet the cells by centrifuging in a conical tube for 5 min at 200 g, and resuspend in Cell Culture media.
- b. Determine cell density using a hemocytometer or equivalent.
- c. Infect cells:
 - For a 96 well plate, resuspend cells at a density of 2×10^5 cells/mL. Add 100 μ L of the cell suspension (2×10^4 cells) to each well containing the RVPs and mix gently by pipetting 1-2 times (**see Note 2**).
 - For a 384 well plate, resuspend cells at a density of 3×10^5 cells/mL. Add 30 μ L of the cell suspension (9×10^3 cells) to each well containing the RVPs and mix gently by pipetting 1-2 times (**see Note 2**).
- d. Place plate in a 5% CO₂, 37°C humidified incubator for 72 hours (**see Note 3**).

D. Quantification of SARS-CoV-2 RVP infection using Promega *Renilla-Glo* Luciferase Assay System

- a. All subsequent processing of samples should be done using the Promega *Renilla-Glo* Luciferase Assay System per the manufacturer's instructions. Our recommendations are included below.
- b. For 96-well assay plates:
 - Centrifuge the plates for 5min at 2000rpm to prevent cell loss
 - Carefully aspirate the supernatant with a multichannel pipette
 - Dilute *Renilla-Glo* Assay Substrate into the Assay Buffer at 1:100, add 30uL of the Substrate:Buffer to each well of a 96-well
 - Incubate for 10 minutes in a dark space
 - Detect luminescence using a luminometer
- c. For 384-well assay plates:
 - Centrifuge the plates for 5min at 2000rpm to prevent cell loss
 - Aspirate supernatant and wash with 35uL of PBS
 - Dilute *Renilla-Glo* Assay Substrate into the Assay Buffer at 1:100, add 15uL of the Substrate:Buffer to each well of a 384-well plate
 - Incubate for 10 minutes in a dark space
 - Detect luminescence using a luminometer

Note: Devices used to read luminescence will vary in relative light unit values based on their individual detectors and software, but the signal to background and Z' will be comparable across devices. Signal to background and Z' can be calculated using mock infected cells as the negative control value.

Notes:

1. For maximal infection, do not thaw RVPs at room temperature.
2. The number of cells seeded for infectivity is critical for obtaining maximal luciferase signal. Because infection takes place over the course of 3 days (recommended), small changes in cell density can result in fluctuations in luciferase signal due to the presence of too few cells or over-confluency at the time of assay.
3. Evaporation can often create an edge effect on plates. Be sure that your incubator is properly humidified, that plates are placed within the incubator away from internal air flow, and, if desired, do not include experimental wells on the edges of the plates.

A sample 96-well plate layout is below, with each Ab tested in duplicate at 11 concentrations. The final volume in each well is 200 μ L (50 μ L serially diluted neutralizing antibody or serum, 50 μ L diluted RVPs, and 100 μ L cells).

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