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Neutralization of 293T-hsACE2 cells with SARS-CoV-2 firefly luciferase Reporter Virus Particles

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/sarscov2rvpdocuments/

Required Reagents

- SARS-CoV-2 firefly luciferase Reporter Virus Particles (RVPs) (Integral Cat# RVP-7xxF)
- Clonal 293T-hsACE2 cells (Integral Cat# C-HA102)
- 96 or 384 well plate (white opaque bottom, tissue culture treated)
- Cell Culture media (DMEM + 10% FBS + 10 mM HEPES + 1x Penicillin-Streptomycin) (Corning Cat# 10017CM, Summerlin Cat# SS-100, Corning Cat# 25-060-CI, Corning Cat# 30-002-CI)
- 0.05% Trypsin-EDTA (Corning Cat# 25-052-CI)
- Neutralizing antibody (see below) or serum (provided by the end user)
    - Integral antibody IM-COV2-F06 for Wuhan-hu-1, Alpha, D614G, and select Omicron RVPs*
    - Integral antibody IM-COV2-G06 for Wuhan-hu-1, Alpha, and Delta RVPs
- Luciferase Assay System (Promega #E1500)

**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 fireflies (Photinus pyralis), therefore a reagent compatible with firefly luciferase is required. Detection reagents for Renilla luciferase will not yield a positive result.

A. Neutralizing antibody or serum incubation with RVPs

a. 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.

b. You will need a total volume of 10 µL/well for a 384-well plate or 50 µL/well for a 96-well plate, of your 2X diluted antibody/serum.

B. Thawing, diluting, and plating RVPs

a. 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)**.

b. Gently mix the RVPs tube by inversion.

c. Dilute the recommended amount of RVP per well in Cell Culture media for a total volume of 10 µL/well for a 384-well plate or 50 µL/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. This will be a total volume of 100 µL/well for a 96-well plate or 20 µL/well for a 384-well plate, with the serum/MAb and RVPs now each at 1X concentration.

e. Incubate at 37°C for 1 h.

C. Seeding and infection of 293T-hsACE2 cells (see Note 2)

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 x 10^5 cells/mL. Add 100 µL of the cell suspension (2 x 10^4 cells) to each well containing the RVPs and mix gently by pipetting 1-2 times **(see Note 3)**.
- For a 384 well plate, resuspend cells at a density of 3 x 10^5 cells/mL. Add 30 µL of the cell suspension (9 x 10^3 cells) to each well containing the RVPs and mix gently by pipetting 1-2 times **(see Note 3)**.

d. Place plate in a 5% CO_2, 37°C humidified incubator for 48-72 hours **(see Note 4 and 5)**.

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

a. All subsequent processing of samples should be done using the Promega Luciferase Assay System as 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.
- Equilibrate the 5X lysis buffer to room temperature and then make a 1X solution of lysis buffer using sterile water.
- Carefully aspirate the supernatant with a multichannel pipette.
- Wash cells by carefully adding 100uL of PBS -/- to each well and gently removing as much of the PBS as possible.
- Add 20uL of the lysis buffer to each well of the 96-well assay plate.
- Add 100uL of Luciferase Assay Reagent to each well of the 96-well assay plate.
- Detect luminescence in a luminometer immediately after reagent addition.

c. For 384-well assay plates:

- Centrifuge the plates for 5min at 2000rpm to prevent cell loss.
- Equilibrate the 5X lysis buffer to room temperature and then make a 1X solution of lysis buffer using sterile water.
- Aspirate supernatant and add 35uL of PBS -/-.
- Aspirate leaving 15uL of liquid behind.
- Add 5uL of the lysis buffer to each well of a 96-well and pulse plate in a centrifuge up to 1000rpm
- Add 20uL of Luciferase Assay Reagent to each well of a 96-well and pulse plate in a centrifuge up to 1000rpm

- Detect luminescence in a luminometer immediately after reagent addition.

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. RVPs can also be used to infect attached 293T-hsACE2 cells by directly adding RVPs to a monolayer of cells at 50-60% confluency.
3. The number of cells seeded for neutralization is critical for obtaining maximal luciferase signal. Because infection takes place over the course of 2-3 days, 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.
4. Luciferase signal can be observed as early as 48 hours after infection, but maximal signal is typically observed at 72 hours.
5. 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 µL (50 µL serially diluted neutralizing antibody or serum, 50 µL diluted RVPs, and 100 µL cells).

	Ab conc.	Conc1 **1**	Conc2 **2**	Conc3 **3**	Conc4 **4**	Conc5 **5**	Conc6 **6**	Conc7 **7**	Conc8 **8**	Conc9 **9**	Conc10 **10**	Conc11 **11**	No Ab **12**
Ab #1	**A**												
Ab #1	**B**												
Ab #2	**C**												
Ab #2	**D**												
Ab #3	**E**												
Ab #3	**F**												
Ab #4	**G**												
Ab #4	**H**												