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Neutralization of Bundibugyo *Renilla* Luciferase Reporter Virus Particles using HEK-293T target Cells

Safety note: Bundibugyo RVPs are created using a VSV ΔG pseudotyping system and should be used under BSL2 or enhanced BSL2 laboratory conditions. Use of Bundibugyo RVPs requires infectious agent training. Always wear gloves, eye protection, and a lab coat when handling RVPs. Filtered tips on pipettes are recommended when handling infectious agents. Please see the Bundibugyo virus RVP Safety Data Sheet for full safety information. https://www.integralmolecular.com/sarscov2rvpdocuments/

Required Reagents

- Bundibugyo (BDBV) GFP Reporter Virus Particles (RVPs) (Integral Cat# RVP-1425L)
- Clonal HEK-293T cells
- 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 or serum samples (provided by the end user)
- *Renilla*-Glo Luciferase Assay System (Promega #E2710)

N.B. Catalog numbers correspond to our recommended products. They can be substituted as desired.

N.B. A reagent compatible with Renilla *luciferase is required. Detection reagents for firefly luciferase are incompatible.*

A. Preparation of serum or antibody sample dilutions

a. Prepare dilutions of samples at 2X the desired final concentration in Cell Culture media. Include wells in the plate layout that will not receive antibody or serum as a control for determining maximum infectivity signal.

b. Prepare sufficient volume of 2X sample concentration for 10 µL/well for a 384-well plate.

N.B. Protocol should be optimized for a 96 well plate. Recommended volume of 2X antibody/serum solution is 50 µL/well for a 96-well plate.

B. Incubating RVPs with diluted antibody or serum

a. Retrieve an aliquot of frozen RVPs, place in a 37°C water bath for 3 minutes until just thawed before immediately placing on ice **(see Note 1)**.

b. Gently mix the RVPs tube by inversion.

c. Dilute the recommended volume of RVP per well in Cell Culture media for a total volume of 10 µL/well for a 384-well plate. A recommended volume per well can be found in the Tech Data Sheet for each lot of RVPs or can be determined empirically.

d. Mix the RVPs with the sample dilutions (1:1). This will be a total volume of 20 µL/well for a 384-well plate and a final 1X sample concentration.

e. Incubate at 37°C for 1 hour.

N.B. Protocol should be optimized for a 96-well plate. Recommended volume of 2X antibody/serum solution is 50 µL/well and 50 µL/well of diluted RVPs for a total of 100 µL/well.

C. Infecting HEK-293T cells with RVPs

a. Retrieve a sub-confluent flask of HEK-293T cells and wash with PBS. Lift 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 minutes at 200 rcf followed by resuspension in Cell Culture media.

b. Determine cell density using a hemocytometer or equivalent.

c. Normalize cells to appropriate density and add to plate containing RVPs and samples for infection:

- 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 RVPs and mix gently by pipetting 1-2 times **(see Note 2)**.

d. Place plate in a 5% CO_2, 37°C humidified incubator for 24 hours **(see Note 3)**.

N.B. Protocol should be optimized for a 96 well plate. Recommended cell seeding density is 100 µL at 2 x 10^5 cells/mL (2 x 10^4 cells/well).

D. Quantification of 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 5 minutes at 850 rcf to prevent cell loss.
- Carefully aspirate the supernatant and wash with 100 µL of PBS.
- Dilute *Renilla-Glo* Assay Substrate into the Assay Buffer at 1:100, add 30 µL of the Substrate:Buffer to each well of a 96-well.
- Incubate for 10 minutes protected from light.
- Detect luminescence using a luminometer.

c. For 384-well assay plates:

- Centrifuge the plates for 5 minutes at 850 rcf to prevent cell loss.
- Aspirate supernatant and wash with 35 µL of PBS.
- Dilute *Renilla-Glo* Assay Substrate into the Assay Buffer at 1:100, add 15 µL of the Substrate:Buffer to each well of a 384-well plate.
- Incubate for 10 minutes protected from light.
- Detect luminescence using a luminometer.

N.B. Relative light unit (RLU) measurements are instrument-dependent and may vary due to differences in detector sensitivity, software, instrument model, and calibration. As a result, signal-to-background (S:B) values may differ between instruments. S:B should be calculated using mock-infected cells as the negative control.

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. Small changes in cell density can increase variability in luciferase signal.
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 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**												