Virology at integral molecular

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Neutralization of GFP *Filoviridae* Reporter Virus Particles using Vero target cells

Safety note: Filovirus RVPs for members of the *Filoviridae* family are created using a VSVΔG pseudotyping system and should be used under BSL2 or enhanced BSL2 laboratory conditions. Use of Filovirus 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.

Required Reagents

- Filovirus GFP Reporter Virus Particles (RVPs)
- Vero CCL81 cells (Sigma Cat# 84113001)
- 96 or 384 well plate (white opaque bottom, tissue culture treated or Poly-D-Lysine coated)
- PBS without calcium or magnesium (Hyclone Cat# SH30256.01)
- Cell Culture media (EMEM + 10% FBS) (Corning Cat# 10-009-CVR, Summerlin Cat# SS100)
- 0.05% Trypsin-EDTA (Corning Cat# 25-052-CI)
- Neutralizing antibody or serum (provided by the end user)

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

A. Preparation of serum or antibody dilutions

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. In total, prepare a volume of 10 µL/well for a 384-well plate of 2X antibody/serum solution.

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, and then immediately place on ice **(see Note 1)**.

b. Gently mix the RVP 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. 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 assay.

d. Mix the RVPs with the antibody/serum dilutions (1:1). This will be a total volume of 20 µL/well for a 384-well plate, with the serum/MAb now at a 1X concentration.

e. Incubate at 37°C for 1 hr.

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. Infection of HEK-293T Cells

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 min at 200 x g, and resuspend in Cell Culture media.

b. Determine cell density using a hemocytometer or equivalent.

c. Infect cells:

- 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 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 100µL at 2 x 10^5 cells/mL (2 x 10^4 cells/well).

D. Quantification

a. Acquire GFP signal (ex488/em530) using fluorescent microscopy, flow cytometry, or a high-content imager.

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 GFP signal. Small changes in cell density can result in fluctuations in GFP 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 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												