Protocol F14: Neutralization of Filovirus Ready Reporter Virus™ Infection of Huh-7 Target Cells

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Materials Needed

Plastics

- 96-well V-bottom plate for serial dilutions (Thermo-scientific, Cat# 14-245-72)
- 96-well U-bottom assay plate (Greiner, Cat# 82050-622)
- Lid for 96-well plate or plate seal (Greiner, Cat# 656191)
- Reagent reservoir (Millipore Sigma, Cat# HS120646)

Reagents

- Ready Reporter Virus kit (stored at -80°C, see **Note 1**)
  - Filovirus Ready Reporter Virus particles (“RRV particles”)
  - Huh-7 Ready Reporter Virus target Cells (“Target Cells”)
  - 100X Fusion Signal Enhancer
- HBSS++ (HBSS + 10 mM HEPES + 0.5% BSA) (Corning, Cat# 21-022, Corning, Cat# 45000-690, Sigma-Aldrich, Cat# A9418)

Equipment

- Multichannel pipettes or liquid handling system
- Water bath at 37°C
- Incubator at 37°C
- Compatible flow cytometer for Ex. 409 nm, Em. 447 nm
- Plate shaker (optional)

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

Procedure

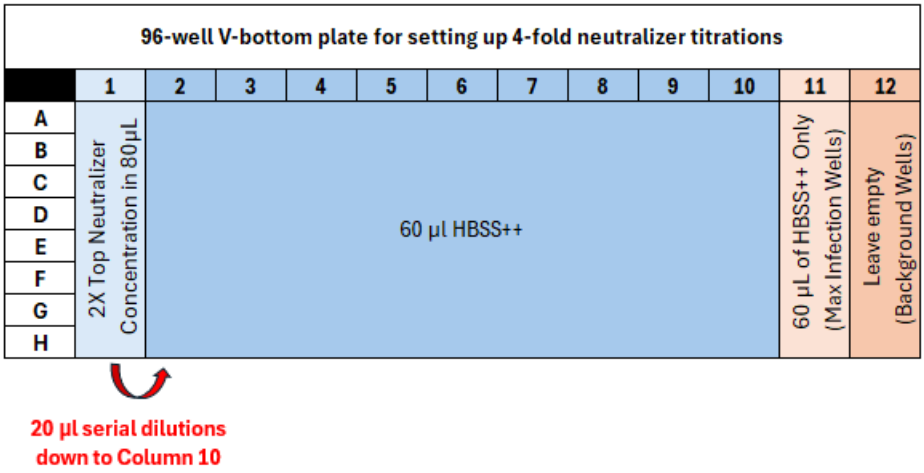
The Filovirus Ready Reporter Virus kit includes non-replicative pseudovirus and target cells pre-loaded with a fluorescent dye to report viral entry and fusion. This procedure is for setting up a neutralization assay using Filovirus Ready Reporter Virus particles ("RRV particles") and Huh-7 Ready Reporter Virus target cells ("Target Cells"). The protocol includes instructions for setting up 10-point, 4-fold neutralization curves in a 96-well plate. Dilution schemes may be adjusted as needed. The protocol and plate layout with example volumes are included below.

Preparation of reagent plates:

Serial dilution of neutralizer samples (Figure 1):

1. Thaw RRV particles for 2-3 minutes in a water bath.
2. In a 96-well V-bottom plate:
    - Column 1: Prepare samples at 2X top concentration in HBSS++ and add 80 µL/well.
    - Columns 2-12: add 60 µL/well HBSS++.
    - From columns 1-10: Perform serial dilutions by transferring 20 µL/well between adjacent columns, mixing several times and changing tips between each column. Exclude column 11 (maximum infectivity) and column 12 (background control) from the serial dilution.

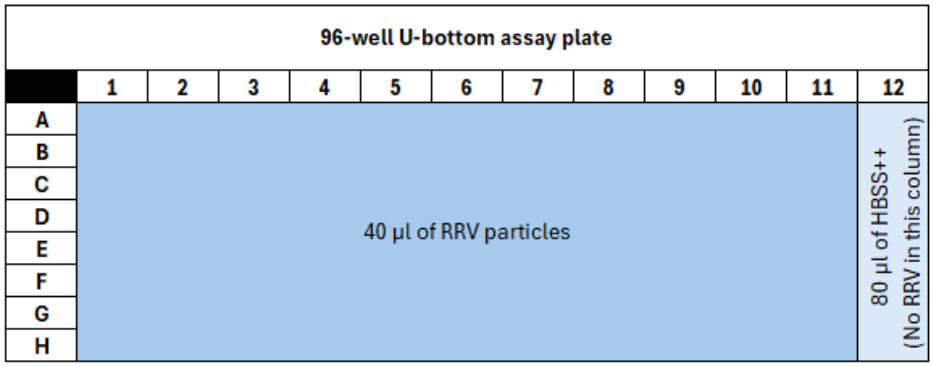
Figure 1. Sample plate layout of serial dilutions.



Preparation of assay plate (Figure 2):

3. In the 96-well U-bottom assay plate, add 80 µL/well of HBSS++ to Column 12. Column 12 will serve as background control.
4. Dilute RRV particles with HBSS++ for a total RRV particle volume of 40 µL/well. Add diluted RRV particles to Columns 1-11 of the assay plate. Recommended RRV particle input volume is given in the Technical Data Sheet of each production lot or can be determined empirically by an Infectivity Assay (see Protocol F13).

Figure 2. Assay plate layout.



Neutralization Set-Up:

5. Transfer 40 µL/well from the corresponding wells of the V-bottom neutralizer sample plate to the matching wells of the U-bottom assay plate, mixing several times.
6. Cover the assay plate with a lid or plate seal and incubate at 37°C for 1 hour.
7. Immediately before incubation of samples with RRV particles is complete, thaw Target Cells in a 37°C water bath. When a small piece of ice remains in the vial, remove Target Cells from the water bath and transfer 500 µL of Target Cells to 5mL of HBSS++ in a 15 mL tube, inverting to mix.
8. Add 35.8 µL of 100X Fusion Signal Enhancer to the Target Cells and invert to mix to produce a suspension of Target Cells with 0.65X Fusion Signal Enhancer in HBSS++.
9. Ensure Target Cells are resuspended and transfer to a reagent reservoir.
10. Add 50 µL/well of Target Cells to the assay plate containing RRV particles and neutralizer samples, mixing several times.
11. Cover with a lid or plate seal and incubate at 37°C for 3 hours. Avoid cells attaching to plates by pipetting to mix or by agitation on a plate shaker at each hour of incubation.

Data Collection:

12. After 3 hours, acquire data by flow cytometry (recommended method, see **Note 2**).
    - If acquiring from the 96-well plate, enable pre-plate shaking function (see **Note 3**).
    - Set up laser lines and PMT to acquire the following emission spectra:

Laser Line	Emission (nm)	Reporter
Violet	447	Infection
Violet	520	No infection

13. To analyze percent infection, background wells can be used to set a fluorescence minus one negative gate based on Em. 447 fluorescent signal (see **Note 4**).

Protocol Notes

Note 1: *Store reagents at -80°C upon receipt. Shelf life of Target Cells may be extended if stored in vapor phase nitrogen. Kit components are single-use only.*

Note 2: *Readout by microscopy with compatible filter sets may be used. Protocols and support of high-content imaging readouts are in development.*

Note 3: *For aspirating from a plate on a flow cytometer, recommended pre-plate shaking settings are 1000 rpm for 60 seconds. We recommend inter-well shakes for 15 seconds at 1000 rpm after each column. We recommend running a cleaning protocol midway through acquiring a plate.*

Note 4: *For analyzing flow cytometry data, we recommend first gating on a healthy singlet cell population.*