Protocol F13: Filovirus Ready Reporter Virus™ Kit Infectivity of Huh-7 Target Cells

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Materials Needed

Plastics

- 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 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-CV, 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 an infectivity assay using Filovirus Ready Reporter Virus particles ("RRV particles") and Huh-7 Ready Reporter Virus target cells ("Target Cells"). Sample volumes for an infectivity assay starting from a stock concentration of RRV particles are included below.

Preparation of Infectivity Assay:

1. Retrieve RRV particles and Target Cells from storage immediately before use.
2. Thaw RRV particles and Target Cells in a water bath. Keep thawed RRV particles on ice and immediately proceed with preparation of Target Cells.

Preparation of Target Cells:

3. 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 5 mL of HBSS++ in a 15 mL tube, inverting to mix.
4. 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++.
5. Ensure cells are resuspended and transfer to a reagent reservoir.

Preparation of Infectivity Assay Plate:

6. In a 96-well U-bottom plate:
   - Add 80 µL of HBSS++ to rows B-H.
   - Add 160 µL of RRV particles to each well of row A.
7. Perform a serial dilution of RRV particles. For a series of two-fold dilutions, transfer 80 µL between adjacent rows, mixing several times and changing tips between each row. Repeat through row G. Leave row H without RRV particles for a background control.
8. Add 50 µL/well of Target Cells to all conditions, including background control wells in row H, mixing several times.

Figure 1. Sample Infectivity Assay plate layout.

	96-well assay plate												
RRV Input Volume (µL)		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
80	**A**	160 µL of RRVs											
40	**B**												
20	**C**												
10	**D**												
5	**E**												
2.5	**F**												
1.25	**G**												
0	**H**	Target Cells only (**No RRV in this row**)											

80 µL serial dilutions through row G

9. Cover the plate and incubate at 37°C for a total of 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:

10. Three hours after infection, 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

11. 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.*