Virology at integral molecular

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Infection of HEK-293T target cells with *Renilla* Luciferase *Filoviridae* Reporter Virus Particles

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 *Renilla* luciferase Reporter Virus Particles (RVPs)
- 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)
- *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 HEK-293T target 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 minutes at 200 rcf followed by resuspension in Cell Culture media.

b. Determine cell concentration using a hemocytometer or equivalent.

B. Thawing, diluting, and plating RVPs for infection of HEK-293T cells

a. Remove an aliquot of frozen RVPs, place in a 37°C water bath for 2 minutes until just thawed, and then immediately place on ice **(see Note 1)**.

b. Gently mix the RVPs tube by inversion.

c. Set up RVP dilutions:

- For 384 well plate, serially dilute the RVPs to a final volume of 20µL per well in Cell Culture media.

N.B. Protocol should be optimized for a 96 well plate. Recommended to serially dilute the RVPs to a final volume of 100µL in Cell Culture media. A suggested plate layout is below, with each sample run in duplicate.

C. Infecting HEK-293T cells with RVPs

a. Normalize cells to appropriate density and add to plate containing RVPs 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)**.

b. 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 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 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 RVP sample in duplicate at 7 concentrations. The final volume in each well is 200 µL (100 µL serially diluted RVPs, plus 100 µL cells).

		Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6	
RVP Input		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
50 µl	A												
25 µl	B												
12.5 µl	C												
6.25 µl	D												
3.12 µl	E												
1.56 µl	F												
0.78 µl	G												
No RVP	H												