

Infection of HEK-293T target cells with GFP *Hantaviridae* Reporter Virus Particles

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

Required Reagents

- Hantavirus GFP Reporter Virus Particles (RVPs)
- 96 or 384 well plate (clear bottom, tissue culture treated, or Poly-D-Lysine coated)
- PBS without calcium or magnesium (Hyclone Cat# SH30256.01)
- 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)

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

A. Preparation of HEK-293T target cells

- 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 centrifugation, and resuspend in Cell Culture media.
- Determine cell concentration using a hemocytometer or equivalent.

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

- 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**).
- Gently mix the RVPs tube by inversion.
- 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 Hantavirus RVPs to a final volume of 100µL in Cell Culture media. A suggested plate layout is below, with each sample run in duplicate.

C. Infection of HEK-293T cells

- Infect cells:
 - For a or a 384 well plate, resuspend cells at a concentration of 3×10^5 cells/mL. Add 30 µL of the cell suspension (9×10^3 cells) to each well containing the RVPs and mix gently by pipetting 3-4 times (**see Note 2**).
- Place plate in a 5% CO₂, 37°C humidified incubator for 24 hours (**see Note 3**).

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