

Neutralization of GFP *Hantaviridae* Reporter Virus Particles using HEK-293T target cells

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)
- 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

- 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.
- 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

- 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**).
- Gently mix the RVP tube by inversion.
- 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.
- 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.
- 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