

Neutralization of Hantaan GFP Reporter Virus Particles using Vero target cells

Safety note: Hantaan RVPs are created using a VSV ΔG pseudotyping system and should be used under BSL2 or enhanced BSL2 laboratory conditions. Use of Hantaan 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. Please see the Hantaan RVP Safety Data Sheet for full safety information. <https://www.integralmolecular.com/sarscov2rvpddocuments/>

Required Reagents

- Hantaan GFP Reporter Virus Particles (RVPs) (Integral Cat# RVP-2350G)
- Vero target cells (ATCC Cat# CCL-81)
- 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 (EMEM + 10% FBS) (Corning Cat# 10-009-CVR, Summerlin Cat# SS-100)
- 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

- a. 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.
- b. 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

- a. 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**).
- b. Gently mix the RVP tube by inversion.
- c. 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.
- d. 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.
- e. 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.

