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Infection of Vero target cells with Hantaan GFP Reporter Virus Particles

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/sarscov2rvpdocuments/

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)

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

A. Preparation of Vero target cells

a. Retrieve a sub-confluent flask of Vero 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.

b. Determine cell concentration using a hemocytometer or equivalent.

B. Thawing, diluting, and plating RVPs for infection of Vero 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 Hantaan 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 Hantaan 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 Vero cells

a. Infect cells:

- For a or a 384 well plate, resuspend cells at a concentration of 3 x 10^5 cells/mL. Add 30 µL of the cell suspension (9 x 10^3 cells) to each well containing the RVPs and mix gently by pipetting 3-4 times (**see Note 2**).

b. Place plate in a 5% CO_2, 37°C humidified incubator for 24 hours **(see Note 3)**.

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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

a. Acquire GFP signal (ex488/em530) using fluorescent microscopy, flow cytometry, or a high-content imager.

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												