

Infection of 293T-hsACE2 cells with SARS-CoV-2 GFP Reporter Virus Particles

Safety note: SARS-CoV-2 RVPs are created using a second-generation lentiviral system and should be used under BSL2 or enhanced BSL2 laboratory conditions. Use of SARS-CoV-2 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. Please see the SARS-CoV-2 RVP Safety Data Sheet for full safety information. <https://www.integralmolecular.com/sarscov2rvpddocuments/>

Required Reagents

- SARS-CoV-2 GFP Reporter Virus Particles (RVPs) (Integral Cat# RVP-7xxG)
- Clonal 293T-hsACE2 cells (Integral Cat# C-HA102)
- 96 or 384 well plate (clear 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)

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

A. Preparation of 293T-hsACE2 cells

- Retrieve a sub-confluent flask of 293T-hsACE2 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 density using a hemocytometer or equivalent.

B. Thawing, diluting, and plating RVPs

- Remove an aliquot of frozen RVPs, place in a 37°C water bath for 2-3 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 96 well plate, serially dilute the SARS-CoV-2 RVPs to a final volume of 100 µL in Cell Culture media per well. A suggested plate layout is below, with each sample run in duplicate
 - For 384 well plate, serially dilute the SARS-CoV-2 RVPs to a final volume of 20 µL in Cell Culture media per well.

[illegible]