



Infection of HEK 293T cells with CHIKV GFP Reporter Virus Particles

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

Required Reagents

- CHIKV GFP Reporter Virus Particles (RVPs) (Integral Cat# RVP-1101G)
- HEK 293T cells
- 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 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 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 CHIKV 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 CHIKV RVPs to a final volume of 20 µL in Cell Culture media per well.

C. Infection of HEK 293T cells

- Infect cells:
 - For a 96 well plate, resuspend cells at a density of 2×10^5 cells/mL. Add 100 µL of the cell suspension (2×10^4 cells) to each well containing the RVPs and mix gently by pipetting 3-4 times (**see Note 2**).



- For a 384 well plate, resuspend cells at a density of 3×10^5 cells/mL. Add 30 μ L 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**).
- b. Place plate in a 5% CO₂, 37°C humidified incubator for 72 hours (**see Note 3**).

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. Because infection takes place over the course of 3 days (recommended), 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 μ L (100 μ L serially diluted RVPs, plus 100 μ L 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 μ L	A												
25 μ L	B												
12.5 μ L	C												
6.25 μ L	D												
3.12 μ L	E												
1.56 μ L	F												
0.78 μ L	G												
No RVP	H												