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Infection of BHK DC-SIGN cells with Dengue or Zika (DENV or ZIKV) *Renilla* luciferase Reporter Virus Particles

Safety note: DENV and ZIKV RVPs are structurally intact virus-like particles, capable of a single round of infection, and should be used under BSL2 or enhanced BSL2 laboratory conditions. Use of DENV or ZIKV 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 Flavivirus RVP Safety Data Sheet for full safety information.
https://www.integralmolecular.com/sarscov2rvpdocuments/

Required Reagents

- DENV or ZIKV Reporter Virus Particles (RVPs) (Integral Cat# RVP-101L-401L, 601L)
- Clonal BHK DC-SIGN cells (Integral Cat# C-BD101)
- pH 8.0 Infection 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-Cl, Corning Cat# 25-025-Cl, Corning Cat# 25-015-Cl)
    - Adjust pH using either NaOH or HCL to a final unit between 7.9-8.1.
- 0.05% Trypsin-EDTA (Corning Cat# 25-052-Ci)
- 96 well plate (black opaque tissue culture-treated) (ThermoFisher Cat# 7805)
- *Renilla* Luciferase Assay System (Promega #E2810)

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

NOTE: The luciferase RVPs contain luciferase from sea pansy (Renilla reniformis), therefore a reagent compatible with Renilla luciferase is required. Detection reagents for firefly luciferase will not yield a positive result.

A. Preparation of BHK DC-SIGN cells

a. Retrieve a sub-confluent flask of BHK DC-SIGN cells and wash with PBS-/-. Lift cells using 0.05% Trypsin-EDTA, end trypsinization using Infection media (double or more the volume of the Trypsin-EDTA added), pellet the cells by centrifugation, and resuspend in Infection media.

b. Determine cell density using a hemocytometer or equivalent.

B. Plating RVPs

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 Footnote 1)**.

b. Gently mix RVPs by inversion.

c. Set up RVP dilutions:

- Place 100uL of Infection Media in all designated wells of the 96-well plate.
- In row A, combine 100uL of RVPs with media and perform a serial 2-fold dilution down the plate. A suggested plate layout is below, with each sample run in duplicate.
- Be sure to include a media only control row with no RVPs.

C. Infection of BHK DC-SIGN cells

a. Infect cells:

- Resuspend cells at a concentration of 3 x 10^5 cells/mL using Infection Media. Add 100 µL of the cell suspension (3 x 10^4 cells) to each well containing the RVPs and mix gently by pipetting 3-4 times **(see Footnote 2)**.

b. Place plate in a 5% CO_2, 37°C humidified incubator for 72 hours **(see Footnote 3)**.

D. Quantification of DENV or Zika infection using Promega *Renilla* Luciferase Assay System

a. All subsequent processing of samples should be done using the Promega *Renilla* Luciferase Assay System as per the manufacturer's instructions. Our recommendations are included below.

- Centrifuge the plates for 10min at 2000rpm to prevent cell loss.
- Carefully aspirate the supernatant with a multichannel pipette
- Prepare 1X *Renilla* Lysis buffer and add 25uL to each well
- Cover the plate with plate sealing tape and shake at medium speed for 15 minutes at room temperature
- Dilute *Renilla* Assay Substrate in the Assay Buffer at 1:100, and add 30uL of the Substrate:Buffer to each well
- Detect luminescence using a luminometer immediately. Delays in reading can increase signal degradation

NOTE: Devices used to read luminescence will vary in relative light unit values based on their individual detectors and software, but the signal to background will be comparable across devices. Signal to background can be calculated using mock infected cells as the negative control value.

Footnotes:

1. For maximal infection, do not thaw RVPs at room temperature.
2. The number of cells seeded for infectivity is critical for obtaining maximal signal. Because infection takes place over the course of 3 days, small changes in cell density can result in fluctuations in 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 and 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												