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Neutralization of Yellow Fever Virus (YFV) *Renilla* luciferase Reporter Virus Particles using BHK DC-SIGN cells

Safety note: YFV 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 YFV RVPs requires infectious agent training. Always wear gloves, eye protection, and a lab coat when using RVPs. Filter tips on pipettes are recommended when handling infectious agents. Please see the Yellow Fever Virus RVP Safety Data Sheet for full safety information. <https://www.integralmolecular.com/sarscov2rvpdocuments/>

Required Reagents

- YFV Reporter Virus Particles (RVPs) (Integral Cat# RVP-1601L and RVP-1603L)
- 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-CI, Corning Cat# 25-025-CI, Corning Cat# 25-015-CI)
 - o 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 (white opaque tissue culture-treated) (Corning Cat# CLS3917)
- *Renilla* Luciferase Assay System (Promega #E2810)
- Neutralizing antibody or serum (provided by the end user)

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 neutralizing antibody or serum dilutions

- a. Prepare dilutions of neutralizing serum or antibody at 2X the desired final concentration in Infection Media. Be sure to include a no antibody condition, which will serve as a control for 100% infectivity.
- b. You will need a total volume of 90 μ L/well of the 2X diluted antibody/serum.

B. Incubating RVPs with diluted antibody or serum

- 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 the RVPs tube by inversion.
- c. Dilute the recommended amount of RVP per well in Infection Media for a total volume of 90 μ L/well. 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 needs.
- d. Mix the RVPs with the antibody/serum dilutions (1:1). This will be a total volume of 180 μ L/well, with the serum/MAb and RVPs now each at 1X concentration.
- e. Incubate at 37°C for 1 h.



C. Infecting BHK DC-SIGN cells with RVPs

- 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.
- c. Infect cells:
 - Resuspend cells at a density of 6×10^5 cells/mL using Infection Media. Add 50 μ L of the cell suspension (3×10^4 cells/well) to each well containing the RVPs and mix gently by pipetting 3-4 times (**see Footnote 2**).
- d. Store plate in a 37°C, 5% CO₂ humidified incubator for 72 hours (**see Footnote 3**).

D. Quantification of YFV 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 Ab tested in duplicate at 11 concentrations. The final volume in each well is 230 μ L (90 μ L serially diluted neutralizing antibody or serum, 90 μ L diluted RVPs, and 50 μ L cells).

Ab conc.		Conc1	Conc2	Conc3	Conc4	Conc5	Conc6	Conc7	Conc8	Conc9	Conc10	Conc11	No Ab
		1	2	3	4	5	6	7	8	9	10	11	12
Ab #1	A												
Ab #1	B												
Ab #2	C												
Ab #2	D												
Ab #3	E												
Ab #3	F												
Ab #4	G												
Ab #4	H												