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SARS-CoV-2 Pseudovirus Neutralization Assay

This protocol applies to following ProSci Pseudovirus products:

SARS-CoV-2 (COVID-19) WT Pseudovirus; Cat. No. 95-200
SARS-CoV-2 (COVID-19) Omicron Variant Pseudovirus; Cat. No. 95-201

Safety Considerations:
SARS-CoV-2 (COVID-19) Pseudovirus particles are based on a lentiviral system that harbors a firefly luciferase as a reporter. The particles are infectious to ACE-2 expressing cells. While the particles are replication defective, they should only be used in Biological Safety Level-2 (BSL-2) laboratory. Researchers should have infectious agents training and should wear lab coat, gloves, and eye protection. Use only filter tips on pipettors.

For complete safety information, please see Safety Data Sheet for Cat. No. 95-200 & Cat. No. 95-201

Materials & Reagents:

- Neutralization and Control Antibodies
- Firefly luciferase reporter pseudovirus of SARS-CoV-2 (ProSci Cat. No. 95-200) or Omicron variant (ProSci Cat. No. 95-201)
- 293T-hsACE2 cells (for example: Integral Cat. No. C-HA101)
- Cell culture medium: DMEM, high glucose, L-glutamine, sodium pyruvate, no phenol red (Biotechne Cat. No. M18650): + 10% Cytiva HyClone™ FetalClone™ II Serum (Fisher Scientific Cat. No. SH3006603) + 1x Penicillin-Streptomycin (Fisher Scientific Cat. No. SV30010)
- Corning® 96-well Solid White Flat Bottom Polystyrene TC-treated Microplates, 20 per bag, with Lid, Sterile (Corning Cat. No. 3917)
- Cytiva HyClone™ Trypsin Protease (Fisher Scientific Cat. No. SH3023601)
- Britelite plus Reporter Gene Assay System (PerkinElmer Cat. No. 6066761)
- Luminometer plate reader

Procedure:
Note: All steps should be carried out in a BSL-2 environment to avoid contamination.

I. **Antibody dilution:**
   1. For the 96-well plate, 1:2 serially dilute the antibodies to a final volume of 50 µL in culture medium per well of each row except the control wells (column 12) containing 50 µL of culture medium (triplicate in column I, II and III) as indicated in the table below.
   2. For each dilution step, mix 6-8 times by pipetting up and down without producing air bubbles.

3. After completing serial dilution, the final 50 µL from the final wells in Column 11 should be discarded.

Example of assay setup on a standard 96 well tissue culture plate:

			Ab serial dilution from Column 1 to Column 11											No Ab
			1	2	3	4	5	6	7	8	9	10	11	12
		A												
Ab-1	I	B	50	50	50	50	50	50	50	50	50	50	50	50
	II	C	50	50	50	50	50	50	50	50	50	50	50	50
	III	D	50	50	50	50	50	50	50	50	50	50	50	50
Ab-2	I	E	50	50	50	50	50	50	50	50	50	50	50	50
	II	F	50	50	50	50	50	50	50	50	50	50	50	50
	III	G	50	50	50	50	50	50	50	50	50	50	50	50
		H												

II. Thawing, diluting, and plating pseudovirus

1. Transfer an aliquot of frozen pseudovirus into a water bath and thaw in a 37°C for 3 minutes and then immediately place on ice. Gently mix the pseudovirus by inverting the tube.
2. Dilute pseudovirus 10 times with culture medium. Add 50 µL of the diluted pseudovirus to each well. Mix 2 times by pipetting up and down without producing air bubbles.

III. Seeding and infection of 293T-hsACE2 cells

1. 293T cells are removed from the plate and suspended in medium in the following way. First, remove the growth medium from the T75 flask. Then, gently rinse cells with 5 mL of PBS and discard the solution. Add 2 mL of trypsin to the flask with 2 minute incubation at 37°C with 5% CO_2 to detach cells.
2. Add 8 mL of cell culture medium to the detached cells and re-suspend cells by pipetting cells up and down up to 10 times with a 10 mL pipette to get a single cell suspension.
3. Centrifuge the cells for 5 minutes at 200 g and re-suspend the cell pellet in 10 mL of cell culture medium.
4. Count cells using a hemocytometer and calculate the cell density.
5. Resuspend cells to a density of 4 x 10^5 cells/mL, then add 100 µL of the cell suspension to each well containing pseudovirus with or without antibodies (4 x 10^4 cells/well) and mix gently by pipetting 2 times. Each well should have 200 µL of solution.
6. Incubate the plate for 48 hours at 37°C with 5% CO_2.

IV. Quantification of pseudovirus infection using Britelite plus Reporter Gene Assay System

1. Remove plates from the incubator and allow cooling to room temperature.
2. Centrifuge the plates at 200 g for 5 minutes.
3. Remove 100 µL of supernatant from each well of culture plates. Each well should have 100 µL of solution left.
4. Perform firefly luciferase assays according to the protocol of Britelite plus Reporter Gene Assay System. Add 100 µL of Britelite plus reagent to each well. Mix gently by pipetting 3 times and immediately place the plate in a luminometer plate reader. Measure luminescence in 2-15 minutes for maximum sensitivity.