Protocols for Antibody Neutralization Assay

1. Seed a 1X gelatin-coated 96-well plate with $1.5x10^4$ 293T-hACE2 cells (CMV-hACE2) per well. Plan to infect this plate 8–12 hours post-seeding.
2. About 1.5 hours prior to infecting cells, begin preparing antibody dilutions in a separate 96-well "setup" plate:
   a) Samples Setting:
   - **Positive control**: Pseudovirus only
   - **Negative control 1**: No pseudovirus
   - **Negative control 2**: Antibody only
   - **Antibody + Pseudovirus**: Pseudovirus + Antibody (with serial dilution)

   b) In the "setup" plate, serially dilute antibody samples with DMEM medium and leave 60 uL diluted antibody in each well.
   For example, start with an initial antibody dilution of 1:10 and do serial 5-fold dilutions.
   c) For Positive control wells and Negative control 2, add 60 ul DMEM medium per well. For Negative control 1 well, add 120 ul DMEM medium per well.

Plate Layout Design:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

3. Dilute virus to a certain concentration (e.g. ~1-2 $x10^6$ IFU/mL). Add 60 ul of diluted virus to all wells containing antibody dilutions and the virus plus cells control wells.
4. Incubate virus and antibody at 37 °C for 1 hr.
5. Carefully add 100 uL mixture from each well of the setup plate containing the antibody and virus dilutions to replace the medium in corresponding wells of the HEK293T-hACE2 cells plate.
6. Add Transplus™ (Alstem, Cat# V050) as described for a final concentration of 1X in each well.

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7. Incubate at 37 °C for 48-60 hours before reading out fluorescence (or luminescence). Count the fluorescence (e.g., GFP) positive the cell in each well, or run FACS to calculate the percentage of fluorescence positive cells in each well. Or measure the luminescence in each well if the psedovirus contains a luciferase gene.
8. Plot the data.

Related Spike (SARS-CoV-2) pseudotyped lentivirus and cell line:

Spike (SARS-CoV-2) pseudotyped GFP lentivirus, Cat# SV01
Spike (SARS-CoV-2) pseudotyped Luciferase-GFP lentivirus, Cat# SV11
HEK293-hACE2 cell line, Cat# AL01

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