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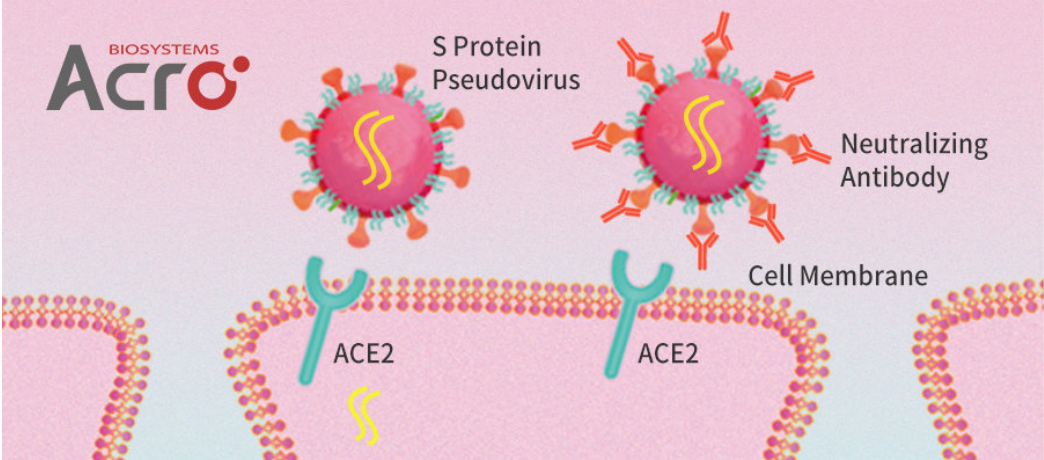
SARS-CoV-2 Spike (WT) Fluc-GFP Pseudovirus uses pseudotyped HIV-1 virus with firefly luciferase and green fluorescent protein (GFP) gene as the backbone and takes SARS-CoV-2 spike protein as its envelope protein. It can effectively infect human ACE2 overexpressing cells and can be used in determining neutralizing antibody titer, screening for inhibitors of the Spike-ACE2 interaction, studying virus invasion and COVID-19 vaccine development.

Mutation(s) compared to wild type surface glycoprotein [severe acute respiratory syndrome coronavirus 2] (Accession # QHD43416.1): N/A.

Pseudovirus Profile

Product description	SARS-CoV-2 Spike (WT) Fluc-GFP Pseudovirus
Backbone	HIV-1
Envelope protein	SARS-CoV-2 Spike Protein (wild type)
Reporter	Firefly luciferase, GFP
Physical appearance	Dark red to dark brown transparent liquid
Storage	-70°C
Transport	Dry ice
Application	Neutralization assay

Schematic Diagram (Neutralization)



Protocol of Pseudovirus Neutralization Assay

- Mix 89% DMEM medium, 10% Fetal bovine serum and 1% Penicillin-Streptomycin to prepare complete DMEM medium.
- Thaw the pseudovirus at room temperature. Dilute the pseudovirus with complete DMEM medium **according to your pre-test results**. In general, we recommend 125-fold dilution if you use the same materials and luminescence meter as those in this protocol (e. g., 20 µL pseudovirus + 2.48 mL complete DMEM medium).
- Dilute your samples with complete DMEM medium in a 96-well white flat bottom plate to reach a volume of 75 µL per well, then add 25 µL pseudovirus suspension per well to reach a final volume of 100 µL per well. Gently flap to mix well. Incubate the plate in a 5% (vol/vol) CO<sub>2</sub>, 37°C incubator for 60 min.
- Digest and resuspend HEK293/Human ACE2 Overexpression Stable Cells (ACROBiosystems, Cat. No. CHEK-ATP042) with complete DMEM medium. Adjust the cell density to 4 ~ 5 × 10<sup>5</sup> cells per milliliter with complete DMEM medium. Seed 100 µL the cell suspension per well into the 96-well plate. Gently flap to mix well. Incubate the plate in a 5% (vol/vol) CO<sub>2</sub>, 37°C incubator for 48 h.
- Prepare the detection reagent (britelite plus Reporter Gene Assay System (PerkinElmer, Cat. No. 6066761)) and balance it to room temperature.
- Take out the 96-well plate and discard 100 µL medium per well. Balance the plate to room temperature for 10 min. Add 100 µL detection reagent and mix well. Incubate for 2 min at room temperature.
- Read the luminescence values (RLU) of the wells with a luminescence meter (PerkinElmer, Cat. No. HH34000000).
- Calculate the inhibition rate with the following formula:

Inhibition rate =  $\left(1 - \frac{X - \overline{CC}}{\overline{VC} - \overline{CC}}\right) \times 100\%$

X: the luminescence value (RLU) of a certain well;

CC, cell control, only cells are added;

$\overline{CC}$ , the mean value of cell control group;

VC, virus control, only cells and pseudovirus are added;

$\overline{VC}$ , the mean value of virus control group.

Protocol of GFP Imaging Assay

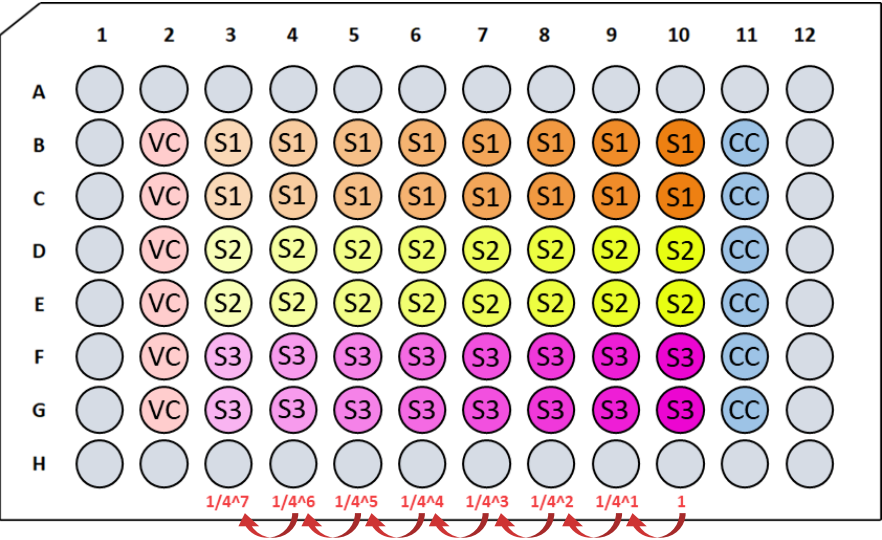
- Mix 89% DMEM medium, 10% Fetal bovine serum and 1% Penicillin-Streptomycin to prepare complete DMEM medium.
- Thaw the pseudovirus at room temperature. Add pseudovirus with a suitable dosage into a transparent 96-well cell culture plate **according to your pre-test results**. In general, the dosage should not be less than 4 µL per well. Then add complete DMEM medium containing your samples or not, to make the total volume is 100 µL per well. The neutralization process can be done based on the “Protocol of Pseudovirus Neutralization Assay”.
- Digest and resuspend HEK293/Human ACE2 Overexpression Stable Cells (ACROBiosystems, Cat. No. CHEK-ATP042) with complete DMEM medium. Adjust the cell density to 4 ~ 5 × 10<sup>5</sup> cells per milliliter with complete DMEM medium. Seed 100 µL the cell suspension per well into the 96-well plate. Gently flap to mix well. Incubate the plate in a 5% (vol/vol) CO<sub>2</sub>, 37°C incubator for 48 h.
- Take out the 96-well plate, discard the medium. Add 30 µL 0.25% Trypsin-EDTA per well, put the plate back to the 5% (vol/vol) CO<sub>2</sub>, 37°C incubator for 2 min.
- Take out the 96-well plate, add 170 µL complete DMEM medium per well to neutralize trypsin.
- Use a pipette to disperse and mix the cells well, pipet 100 µL per well into a well of a transparent 24-well cell culture plate.
- Add 400 µL complete DMEM medium per well into the 24-well plate. Gently flap to mix the reagents and cells in the 24-well plate well. Put the plate in a 5% (vol/vol) CO<sub>2</sub>, 37°C incubator for another 48 h.
- Take out the 24-well plate, and take GFP pictures with a fluorescence microscope. The recommended enlargement factor of the objective is 20x.

Notice:

- Please refer to the “Experimental Data” part to figure out the GFP positive rate with this virus dosage. If you want a higher GFP positive rate, or you use another cell line, please increase the virus dosage.
- If you design to do the infection in a larger system (e. g., in a 6-well plate), please casrry out a pre-test first in a 96-well plate to confirm the suitable virus dosage.

Sample dilution with a 96-well plate

Mutiple layouts and dilution strategies are acceptable for pseudovirus neutralization assay. There is an example of “3 samples & 4-fold dilution”:



- a. Add 200  $\mu$ L PBS per well into the gray wells to reduce evaporation.
- b. Add 75  $\mu$ L medium into well B2-G9, 100  $\mu$ L medium into well B11-G11.
- c. Add 100  $\mu$ L samples diluted by medium into well B10-G10. The concentration of the sample should be 4/3 fold of the first expected gradient.
- d. Pipet 25  $\mu$ L with a multichannel pipettor from each of well B10-G10, into well B9-G9, do blowing-suction for 10 times.
- e. Pipet 25  $\mu$ L from well B9-G9, into well B8-G8, do blowing-suction for 10 times. The rest can be done in the same manner till well B3-G3.
- f. Pipet and discard 25  $\mu$ L from well B3-G3.
- g. Add 25  $\mu$ L pseudovirus dilution per well into well B2-G10, Gently flap to mix well, and go ahead with the following steps.

Notice

Basic advice

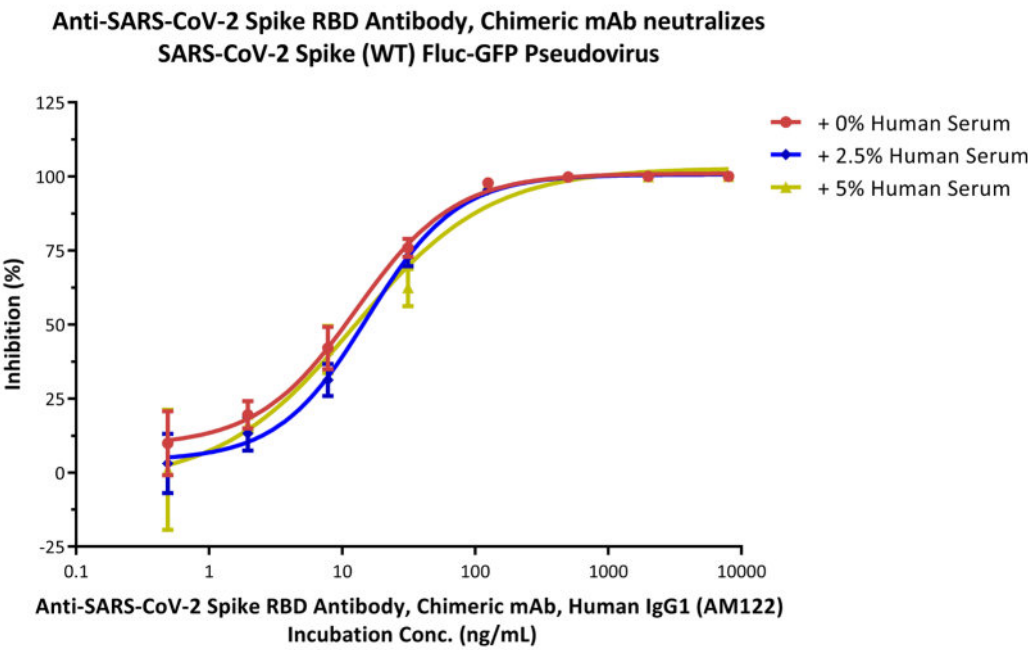
- a. Though pseudovirus particles has no pathogenicity and cannot replicate, the assays should be carried out carefully in a Biosafety Level 2 or higher-level laboratory with a biosafety cabinet.
- b. Serum samples from animals or humans should be inactivated in a water bath at 56°C for 30 min before being tested.
- c. Please avoid freezing and thawing, which would influence the titer of the pseudovirus.
- d. The product is for **Research Use Only**.

Optimal pseudovirus dosage / Reporter choice

- e. Firefly luciferase is an ideal reporter for quantitative experiment because of its high sensitivity. If you plan to use GFP for imaging, more pseudovirus dosage and more incubation time should be used **according to your pre-test results**. Please contact our technical support team for more information.
- f. The luminescence value (RLU) could be influenced by multi-factors. If **cells, detection reagent or luminescence meter** other than those in this protocol is used, a pre-test should be carried out to determine an appropriate pseudovirus dosage.
- g. According to the Data Sheet of the detection reagent, the values should be read in 5 min after addition of the detection reagent and the temperature should be around 22°C. At the same time, as FBS and phenol red show impact on the light output, discard all medium, then add 100  $\mu$ L PBS and 100  $\mu$ L detection reagent if you need a higher luminescence value (RLU).

Experimental Data

Bioactivity - Virus based assay

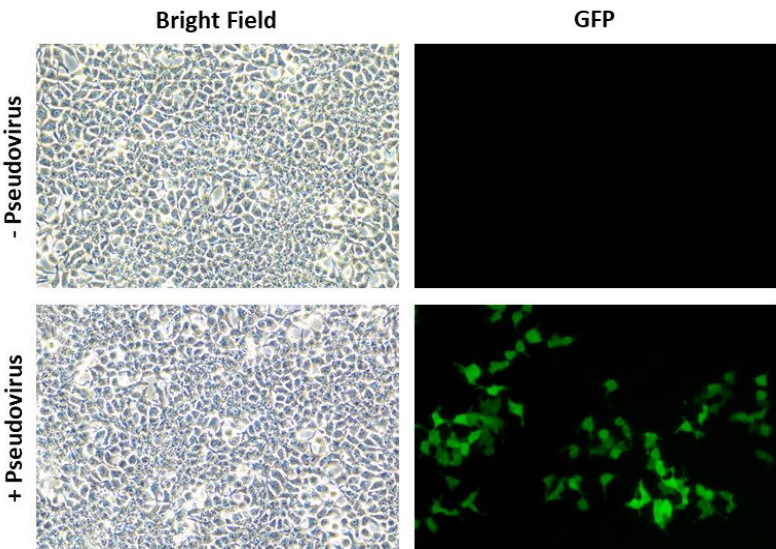


SARS-CoV-2 Spike (WT) Fluc-GFP Pseudovirus (Cat. No. PSSW-HLGB001) is suitable for pseudovirus-antibody (ACROBiosystems, Cat. No. S1N-M122) neutralization assay. Human serum showed limited interference to this assay. The values of absolute IC<sub>50</sub> for this effect are 7.549-15.43 (0% human serum), 8.773-25.70 (2.5% human serum) and 10.08-20.03 (5% human serum) ng/mL (Routinely tested).

Notice:

- a. All data above represent the general characters of this product. Please check the COA of a certain lot to find the exact titer and signal intensity.
- b. For help, more information and protocols of related assays, please contact our technical support team at: [TechSupport@acrobiosystems.com](mailto:TechSupport@acrobiosystems.com).

HEK293/Human ACE2 Stable Cell Line expresses GFP after SARS-CoV-2 Spike (WT) Fluc-GFP Pseudovirus infection



SARS-CoV-2 Spike (WT) Fluc-GFP Pseudovirus (Cat. No. PSSW-HLGB001) can transfect functional GFP gene into HEK293/Human ACE2 Overexpression Stable Cell Line (ACROBiosystems, Cat. No. CHEK-ATP042) (Routinely tested).