ReVacc Scientific
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Protocols for Pseudotyped Virus Titration and Neutralization Assay

We suggest you titrate our product in your system prior to the neutralization assay or any formal assays

Reagents required, but not included

- Target cell line:
  - Vero E6 or cell line expressing ACE2 for SARS, MERS or SARS-CoV-2 pseudovirus
  - Vero for Ebola, Marburg, Lassa, Zika, Japanese Encephalitis, Herpes pseudovirus
  - 293T for Avian Influenza pseudovirus
  - Huh-7 for Hepatitis C pseudovirus
- Cell culture medium, such as cEMEM for Vero E6 or Vero; cDMEM for 293T or Huh-7
- Trypsin-EDTA (0.25%), phenol red
- Luciferase Cell Culture Lysis 5X Reagent (Promega, Catalog # E1531)
- Luciferase Assay System (Promega, Catalog # E1500)

Equipment required

- Vortex
- Water Bath
- Microplate shaker
- Cell culture 37°C, CO_2 incubator
- Luminescence reader for luciferase signal

Titration Protocol

(96-well cell culture plate)

Steps:

1. Thaw the pseudotyped virus at 37°C water bath. Vortex prior to use. Recommend to aliquot 20 µl/vial for future use as the multiple freeze-thaw cycles will significantly reduce its sensitivity. Only one freeze-thaw cycle is recommended.
2. Add 100 µl/well of diluted pseudotyped virus by starting 10-fold dilution for VSV pseudovirus or 2-fold dilution for Lenti-pseudovirus. Duplicate each well. Set a negative control (untreated control, UC) by using 100 µl medium to replace pseudovirus.
3. Add 100 µl medium/well to replace the sample volume as used for later neutralization assay
4. Add 100 µl trypsin-treated and washed cells for 50,000-100,000 per well of 96-well plate.
5. Incubate 1 day at 37°C CO_2 incubator.
6. Next day, remove cell medium and add 60 µl 1X Luciferase Cell Culture Lysis reagent. Put the plate onto a shaker for 40 minutes at 700 rpm speed
7. Transfer 20 µl cell lysate to white plate (Greiner Bio-One, Catalog # 655075) for luminescence measurement (RLU) by following manufacture protocol of Luciferase Assay System (Promega, Catalog # E1500)

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Neutralization Assay Protocol (2-Day Assay)

(96-well cell culture plate)

Steps:

1. Prepare your samples at 100 µl/well in a 96-well tissue culture plate with at least one duplicate (more duplicate, more accurate for results).
2. Thaw and vortex pseudovirus. Add 100 µl/well diluted pseudovirus. The dilution of pseudotyped virus is based on the titration in your system. It might vary in different systems, therefore we strongly suggest you titrate the product in your system prior to test your samples. The recommended dilution fold is where pseudovirus generate 100 - 1,000 fold higher than untreated cells (background). Set a UC control without pseudovirus/sample (200 µl medium) and another control without sample (100 µl medium + 100 µl diluted pseudovirus).

Note: For antibody, we suggest to start 50 µg/ml with 7 serial 5-fold dilution in cell culture medium, or start 100 µg/ml if you think your sample might not be potent. For serum or plasma, 100-fold dilution to start is recommended or 20-fold if you think your sample might not be potent. Each well volume is 200 µl combining sample and diluted pseudovirus, when you calculate your sample concentration.

3. Incubate 1 hour at 37°C CO_2 incubator.
4. Add 100 µl trypsin-treated and washed cells for 50,000-100,000 per well.
5. Incubate 1 day at 37°C CO_2 incubator. Lyse cell next day and read RLU as above-mentioned.

Neutralization Assay Protocol with Reduced Volume (3-Day Assay)

(Most cases for Lenti-Pseudotyped virus to save your sample and pseudovirus)

(96-well cell culture plate)

Steps:

1. Prepare your samples at 10 µl/well with serial dilution in a 96-well tissue culture plate with at least one duplicate (more duplicate, more accurate for results).
2. Thaw and vortex pseudovirus. Add 40 µl/well diluted pseudovirus as above-mentioned. Set a UC control without pseudovirus/sample (50 µl medium) and another control without sample (10 µl medium + 40 µl diluted pseudovirus).

Note: Each well volume is 50 µl combining sample and diluted pseudovirus, when you calculate your sample concentration.

3. Incubate 1 hour at 37°C CO_2 incubator.
4. Add 25 µl trypsin-treated and washed cells for 10,000 per well.
5. Next day, add 150 µl fresh medium for 16-24 hours culture
6. Lyse cell next day and read RLU as above-mentioned.