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Antibody neutralization assay with SARS-CoV-2 and SARS-CoV pseudovirus

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MATERIALS TEXT

- pMDLg/pRRE: Addgene, #12251.
- pRSV-Rev: Addgene, #12253.
- pHIV-luc-ZsGreen: Addgene, #39196.
- Lenti-X^{TM}p24 Rapid Titre Kit: Takara Bio, #632200.
- Lipofectamine 2000 reagent: Invitrogen, 11668019.
- Opti-MEM Reduced Serum Medium: Gibco, 31985070
- StemPro Accutase Cell Dissociation Reagent: Gibco, A1110501
- DMEM cell transfection medium: DMEM (high glucose, Hyclone, SH30022.01) + 10% heat-inactivated FBS (Gibco)
- DMEM cell culture medium: DMEM (high glucose, Hyclone, SH30022.01) + 10% heat-inactivated FBS (Gibco) + 1% Penn-Strep (Gibco, 15140163)

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- 96-well Flat Clear Bottom Black Polystyrene TC-treated Microplates: Corning, 3904
- 5x Passive Lysis Buffer: Promega, E1941
- Luciferase assay system: Promega, E1510
- StemPro Accutase Cell Dissociation Reagent: Gibco, A1110501
- CHO-ACE2 cell culture medium: DMEM (high glucose, Hyclone, SH30022.01) + 10% heat-inactivated FBS (Gibco) + 1% MEM NEAA (Gibco, 11140050) (Add G418 at 0.5 mg/ml (Gibco 10131027) to maintain the CHO-ACE2 cells.)

SAFETY WARNINGS

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Generation of pseudovirus particles

1 Day 1: Transfection of HEK293T cells to generate pseudovirus.

1.1 Dissociate the HEK293T cells with StemPro Accutase Cell Dissociation Reagent and resuspend 5 x 10^6 cells in 9ml DMEM cell transfection medium.

1.2 Prepare the transfection reagent mix in Opti-MEM Reduced Serum Medium.
- Reagent A: 0.5 ml Opti-MEM Reduced Serum Medium containing 3 µg pMDLg/pRRE (Addgene) + 1.5 µg pRSV-Rev (Addgene) + 3 µg pTT5LnX-coV-SP (from Dr. Brendon John Hanson, DSO Singapore, for SARS-CoV-2 spike) or 3 µg pXJ3'-S (from Prof. Yee-Joo TAN, IMCB & NUS, Singapore, for SARS-CoV spike) + 6 µg pHIV-Luc-ZsGreen (Addgene).
- Reagent B: 0.5 ml Opti-MEM Reduced Serum Medium + 30 µl Lipofectamine 2000 reagent, incubate at room temperature for 5 minutes.
- Add Reagent B to Reagent A, gently mix and incubate at room temperature for 20 minutes.

1.3 Add the transfection mix (~1 ml) from **Step 1.2** to the HEK293T cell suspension prepared in **Step 1.1** and mix gently before transfer to a T25 tissue culture flask.

1.4 6 hours post transfection, gently remove all medium and replace with 10 ml of fresh DMEM cell culture medium and continue to culture at 37°C incubator for 3 days.

2 Day 4: Harvest the viral supernatant.

2.1 Harvest culture supernatant that contains the pseudovirus from **Step 1** using 10 ml Syringe and 0.45 µm filter unit and collect the filtered viral supernatant in a 15 ml Falcon tube, the resulting volume should be ~9.5ml.

2.2 Keep 10 µl of filtered viral supernatant for determining the viral titre using Lenti-X™ p24 Rapid Titre Kit in **Step 3**.

2.3 Aliquot the filtered viral supernatant and store at -80°C freezer.

3 Day 4: Determine the viral titer using the Lenti-X™ p24 Rapid Titre Kit.

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3.1 Prepare 10x serial dilution of the 10 µl of filtered viral supernatant from **Step 2.2** in DMEM cell culture medium to achieve 10x, 100x, 1000x, 10000x, and 100000x dilutions.

3.2 Perform ELISA using the Lenti-X p24 Rapid Titre Kit with 1000x, 10000x, 100000x dilutions of the filtered viral supernanant prepared in **Step 3.1** following the manufacturer's instructions.

3.3 Calculate the amount of p24 according to the manufacturer's instructions and back calculate the pseudovirus titer in the original viral supernatant.

Antibody neutralization assay

4 **Day 5: Seed cells for neutralization assay** (at least 3 hours before **Step 5**).

4.1 Dissociate CHO-ACE2 cells (from Prof. Yee-Joo Tan, IMCB, A*STAR) with StemPro Accutase and resuspend cells in CHO-ACE2 cell culture medium to make 0.32 x 10^6 cells per ml cell suspension.

4.2 Seed the cells to 96-well Flat Clear Bottom Black Polystyrene TC-treated Microplates (Corning, 3904) at 32000 cells per well in 100 µl.

4.3 Incubate the plates in 37°C, 5% CO_2 incubator for 3-4 hours.

5 **Day 5: Mix the pseudovirus with potential neutralizing antibodies**.

5.1 In a 96-well tissue culture plate, prepare a serial dilution of each anti-SARS-CoV-2 Spike RBD IgG or Fab antibodies using CHO-ACE2 cell culture medium (total 7 dilutions with 4-fold dilutions, each dilution in 90µl for the triplicate-well experiment).

5.2 Thaw aliquots of frozen SARS-CoV-2 or SARS-CoV pseudovirus supernatant and dilute the pseudovirus supernatant to 0.48 ng/µl of p24 using CHO-ACE2 cell culture medium, hence the amount of virus particles in every 25 µl of diluted pseudovirus supernatant will be equivalent to 12ng of p24. Aliquot 90 µl of the diluted pseudovirus supernatant to each well of a new 96-well tissue culture plate.

5.3 Transfer 90 µl of each diluted antibody samples from **Step 5.2** to each well of90µl ofdiluted pseudovirus supernatant in **Step 5.1**, pipette to mix. Include control wells without adding antibodies (the "virus only control").

5.4 Incubate the plate of pseudovirus-antibody mixtures at 37°C incubator for 1 hour.

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6 **Day 5: Add pseudovirus-antibody mixture to cells**.

6.1 Remove culture medium from the wells seeded with CHO-ACE2 cells in **Step 4**.

6.2 Add 50 µl of pseudovirus-antibody mixture from **Step 5** to each well of CHO-ACE2 cells, in triplicate. Add 50 µl of CHO-ACE2 cell culture medium to the respective wells of CHO-ACE2 cells ("No virus control"), in triplicate.

6.3 Incubate the plate at 37°C incubator for 1 hour to allow pseudovirus infection.

6.4 After 1-hour incubation, take out the plate from incubator, and top-up each well with 150 µl of CHO-ACE2 cell culture medium and continue to incubate the plate at 37°C incubator for additional 48 hours.

7 **Day 7: Lyse cells for luciferase assay**.

7.1 Remove all culture medium from each well of the plates from **Step 6**.

7.2 Wash the cells twice with 150 µl sterile PBS.

7.3 Remove PBS from the second wash, and add add 20 µl of 1x Passive lysis buffer (Promega, E1941, dilute 5x Passive lysis buffer using sterile H_2O) to each wells.

7.4 Incubate the plate at 37°C with gentle shaking at 400rpm for 30 minutes to lyse the cells.

8 **Day 7: Read luciferase activity**.

8.1 While waiting for the cell lysis at **Step 7.4**, freshly prepare Luciferase Assay Reagent by adding Luciferase Assay Buffer (10 ml per vial) to the vial of lyophilized Luciferase Assay Substrate ("Luciferase Assay System", Promega, E1510).

8.2 Read the luciferase activity using the Promega GloMax Plate Reader (choose built-in protocol "Luciferase Assay System").

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