**Pseudotyped virus protocol**

1. **SARS-CoV-1, SARS-CoV-2, MERS pseudotyped viruses (PSVs) production**
* SARS1, SARS2 and VSV-G are MLV-based retrovirus, while MERS uses lentivirus system.
* Seed HEK293T cell to 6-well plate (1.25 million/well) or 10cm dish (7.5million cells/dish) one day before transfection.
* Gently aspirate media, add 2 ml (6-well plate) or 12 mL (10cm dish) fresh DMEM (Gibco, 10569010) complete medium (with 10% Heat Inactivated FBS and 1% P/S) to 6-well plate or 10cm dish before transfection.
* Prepare a mixture of the 3 transfection plasmids (all plasmids are Amp resistant):

**a) Envelope**:

SARS2-D18: 0.5ug for 6-well plate; 2.5ug for 10cm dish

SARS1-D28: 1ug for 6-well plate; 5ug for 10cm dish

VSV-G (Addgene# 8454): 1ug for 6-well plate; 5ug for 10cm dish

MERS-D12: 1ug for 6-well plate; 5ug for 10cm dish

**b) Gag/Pol** (MLV backbone, Addgene# 14887):

 SARS2: 2.5 ug for 6-well plate; 12.5 ug for 10cm dish

 SARS1: 1.5 ug for 6-well plate; 7.5 ug for 10cm dish

 VSV-g: 2ug for 6-well plate; 10ug for 10cm dish

 MERS-D12: 1.5 ug for 6-well plate; 7.5 ug for 10cm dish [use DR8.2 instead (Addgene #12263)]

**c) MLV luciferase** (MLV addgene#18760, IRES changed to CMV promoter):

 SARS2: 2ug for 6-well plate; 10ug for 10cm dish

 SARS1: 2ug for 6-well plate; 12.5ug for 10cm dish

 VSV-G: 2ug for 6-well plate; 10ug for 10cm dish

 MERS: 2ug for 6-well plate; 12.5ug for 10cm dish (use lentivirus plasmid pBOBI-firefly instead)

 **Note: VSVG PSVs should infect both WT HeLa and HeLa-ACE2 cells; SARS-CoV-1**

**and SARS-CoV-2 PSVs can only infect HeLa-ACE2 cells; MERS PSV only infects HeLa-DPP4.**

* I use Lipo2000 from Invitrogen to do the transfection (1ug plasmids/ 2.5ul lipo2000). You can use any other transfection reagents according to manufacturer’s instructions.
* Gently aspirate media, add 3ml or 20 mL fresh DMEM complete medium 12-16 hours post transfection to each well of 6-well plate or 10cm dish respectively.
* Collect the supernatants (PSVs) 48 hours post transfection, aliquot and freeze at -80 for long-term storage.

Option: You can add another 20mL fresh DMEM complete medium, incubate for 24 hours and collect PSVs again (72 hours post transfection).

1. **PSV infection assay (Modified from Elise’s HIV neutralization protocol)**

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| * Serial dilution of antibody or serum with a suitable starting concentration from Row A to H in 96-well plate.
* Transfer 25ul of diluted antibody or sera to each well of 96-well half area well plate (Corning® 96 Half Area, # 3688)
* Add 25ul virus to columns 1-11, and 25ul fresh medium to column 12 (Cells only control).
* Spin down and incubate at incubator for 1hour.
* Prepare HeLa-ACE2 cells in the meanwhile.
* Count and dilute cells to 200,000/ml.
* Add DEAE-dextran (Stock 10mg/ml, 500 X, Sigma, # 93556-1G) to the HeLa-ACE2 cells at a final concentration of 20ug/ml.
* Directly add 50ul of cells to the Ab/Virus or Serum/Virus mixture into 96-well plate.
* Incubate 48 hours at the incubator.
* Read the plate
1. Aspirate medium and add 30/ul of 1 X lysis buffer (25mM Gly-Gly pH 7.8, 15mM MgSO4, 4mM EGTA, 1% Triton X-100, you can make 10X stock and store at 4 degree)
2. Shake the plate for 20 mins to ensure total cell lysis
3. Prepare luciferase substrate (Dilute Bright-glo 5-fold in 1 x lysis buffer)
4. Add 30ul diluted Bright-glo substrate (Promega, #E2650)
5. Luciferase intensity was read on a luminometer

Layout template.% Neutralization=$100\*(1-\frac{RLU of sample-Average RLU of CC}{Average RLU of VC-Average RLU of CC}$)Note:If you still have questions, please refer to the Protocol for Neutralizing Antibody Assay for HIV-1 in TZM-bl Cells. You can find the equation to calculate % neutralization of serum or antibody.<https://www.hiv.lanl.gov/content/nab-reference-strains/html/Protocol-for-Neutralizing-Antibody-Assay-for-HIV-1-in-TZMbl-cells_Nov2018.pdf><https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4040342/>Sequences**SARS-CoV1 spike full-length:**ATGTTCATCTTCCTGCTGTTCCTGACCCTGACCTCTGGCTCTGACCTGGACAGGTGTACCACCTTTGATGATGTCCAGGCTCCAAACTACACCCAACACACCTCCAGTATGAGGGGAGTCTACTACCCTGATGAGATTTTCAGGTCTGACACCCTCTACCTGACCCAGGACCTGTTCCTGCCATTCTACAGCAATGTGACAGGCTTCCACACCATCAACCACACCTTTGACAACCCTGTGATTCCATTCAAGGATGGCATCTACTTTGCTGCCACAGAGAAGAGCAATGTGGTGAGGGGCTGGGTGTTTGGCAGCACAATGAACAACAAGAGCCAGTCTGTGATTATCATCAACAACAGCACCAATGTGGTGATTAGGGCTTGTAACTTTGAACTGTGTGACAACCCATTCTTTGCTGTGAGCAAGCCTATGGGCACCCAGACCCACACAATGATTTTTGACAATGCCTTCAACTGTACCTTTGAATACATCTCTGATGCCTTCTCCCTGGATGTGTCTGAGAAGTCTGGCAACTTCAAACACCTGAGGGAGTTTGTGTTCAAGAACAAGGATGGCTTCCTCTATGTCTACAAGGGCTACCAACCAATTGATGTGGTGAGGGACCTGCCATCTGGCTTCAACACCCTGAAACCAATCTTCAAACTGCCACTGGGCATCAACATCACCAACTTCAGGGCTATCCTGACAGCCTTCAGCCCTGCCCAGGACACCTGGGGCACCTCTGCTGCTGCCTACTTTGTGGGCTACCTGAAACCAACCACCTTTATGCTGAAATATGATGAGAATGGCACCATCACAGATGCTGTGGACTGTAGCCAGAACCCACTGGCTGAACTGAAATGTTCTGTGAAGTCCTTTGAGATTGACAAGGGCATCTACCAGACCAGCAACTTCAGGGTGGTGCCATCTGGAGATGTGGTGAGGTTTCCAAACATCACCAACCTGTGTCCATTTGGAGAGGTGTTCAATGCCACCAAGTTTCCATCTGTCTATGCCTGGGAGAGGAAGAAGATTAGCAACTGTGTGGCTGACTACTCTGTGCTCTACAACAGCACCTTCTTCAGCACCTTCAAGTGTTATGGAGTGTCTGCCACCAAACTGAATGACCTGTGTTTCAGCAATGTCTATGCTGACTCCTTTGTGGTGAAGGGAGATGATGTGAGACAGATTGCCCCTGGACAAACAGGAGTGATTGCTGACTACAACTACAAACTGCCTGATGACTTTATGGGCTGTGTGCTGGCTTGGAACACCAGGAACATTGATGCCACCAGCACAGGCAACTACAACTACAAATACAGATACCTGAGACATGGCAAACTGAGACCATTTGAGAGGGACATCAGCAATGTGCCATTCAGCCCTGATGGCAAGCCATGTACTCCTCCTGCCCTGAACTGTTACTGGCCACTGAATGACTATGGCTTCTACACCACCACAGGCATTGGCTACCAACCATACAGGGTGGTGGTGCTGTCCTTTGAACTGCTGAATGCCCCTGCCACAGTGTGTGGACCAAAACTGAGCACAGACCTGATTAAGAACCAGTGTGTGAACTTCAACTTCAATGGACTGACAGGCACAGGAGTGCTGACACCATCCAGCAAGAGGTTCCAACCATTCCAACAGTTTGGCAGGGATGTGTCTGACTTCACAGACTCTGTGAGGGACCCAAAGACCTCTGAGATTCTGGACATCAGCCCATGTTCCTTTGGAGGAGTGTCTGTGATTACACCTGGCACCAATGCCTCCTCTGAGGTGGCTGTGCTCTACCAGGATGTGAACTGTACTGATGTGAGCACAGCCATCCATGCTGACCAACTTACACCTGCCTGGAGGATTTACAGCACAGGCAACAATGTGTTCCAGACCCAGGCTGGCTGTCTGATTGGAGCAGAGCATGTGGACACCTCCTATGAGTGTGACATCCCAATTGGAGCAGGCATCTGTGCCTCCTACCACACAGTGTCCCTGCTGAGGAGCACCAGCCAGAAGAGCATTGTGGCTTACACAATGAGTCTGGGAGCAGACTCCAGCATTGCCTACAGCAACAACACCATTGCCATCCCAACCAACTTCAGCATCAGCATCACCACAGAGGTGATGCCTGTGAGTATGGCTAAGACCTCTGTGGACTGTAATATGTATATCTGTGGAGACAGCACAGAGTGTGCCAACCTGCTGCTCCAATATGGCTCCTTCTGTACCCAACTTAACAGGGCTCTGTCTGGCATTGCTGCTGAACAGGACAGGAACACCAGGGAGGTGTTTGCCCAGGTGAAGCAGATGTATAAGACACCAACCCTGAAATACTTTGGAGGCTTCAACTTCAGCCAGATTCTGCCTGACCCACTGAAACCAACCAAGAGGTCCTTCATTGAGGACCTGCTGTTCAACAAGGTGACCCTGGCTGATGCTGGCTTTATGAAGCAATATGGAGAGTGTCTGGGAGACATCAATGCCAGGGACCTGATTTGTGCCCAGAAGTTCAATGGACTGACAGTGCTGCCTCCACTGCTGACAGATGATATGATTGCTGCCTACACAGCAGCCCTGGTGTCTGGCACAGCCACAGCAGGCTGGACCTTTGGAGCAGGAGCAGCCCTCCAAATCCCATTTGCTATGCAGATGGCTTACAGGTTCAATGGCATTGGAGTGACCCAGAATGTGCTCTATGAGAACCAGAAGCAGATTGCCAACCAGTTCAACAAGGCTATCAGCCAGATTCAGGAGTCCCTGACCACCACCAGCACAGCCCTGGGCAAACTCCAAGATGTGGTGAACCAGAATGCCCAGGCTCTGAACACCCTGGTGAAGCAACTTTCCAGCAACTTTGGAGCCATCTCCTCTGTGCTGAATGACATCCTGAGCAGACTGGACAAGGTGGAGGCTGAGGTCCAGATTGACAGACTGATTACAGGCAGACTCCAATCCCTCCAAACCTATGTGACCCAACAACTTATCAGGGCTGCTGAGATTAGGGCATCTGCCAACCTGGCTGCCACCAAGATGAGTGAGTGTGTGCTGGGACAAAGCAAGAGGGTGGACTTCTGTGGCAAGGGCTACCACCTGATGAGTTTTCCACAGGCTGCCCCTCATGGAGTGGTGTTCCTGCATGTGACCTATGTGCCAAGCCAGGAGAGGAACTTCACCACAGCCCCTGCCATCTGCCATGAGGGCAAGGCTTACTTTCCAAGGGAGGGAGTGTTTGTGTTCAATGGCACCTCCTGGTTCATCACCCAGAGGAACTTCTTCAGCCCACAGATTATCACCACAGACAACACCTTTGTGTCTGGCAACTGTGATGTGGTGATTGGCATCATCAACAACACAGTCTATGACCCACTCCAACCTGAACTGGACTCCTTCAAGGAGGAACTGGACAAATACTTCAAGAACCACACCAGCCCTGATGTGGACCTGGGAGACATCTCTGGCATCAATGCCTCTGTGGTGAACATCCAGAAGGAGATTGACAGACTGAATGAGGTGGCTAAGAACCTGAATGAGTCCCTGATTGACCTCCAAGAACTGGGCAAATATGAACAATACATCAAGTGGCCATGGTATGTGTGGCTGGGCTTCATTGCTGGACTGATTGCCATTGTGATGGTGACCATCCTGCTGTGTTGTATGACCTCCTGTTGTTCCTGTCTGAAAGGAGCCTGTTCCTGTGGCTCCTGTTGTAAGTTTGATGAGGATGACTCTGAACCTGTGCTGAAAGGAGTGAAACTGCACTACACCTAA**SARS-CoV2 Spike full-length:**atgtttgtgttcctggtgctgctgccactggtgtccagccagtgtgtgaacctgaccaccaggacccaacttcctcctgcctacaccaactccttcaccaggggagtctactaccctgacaaggtgttcaggtcctctgtgctgcacagcacccaggacctgttcctgccattcttcagcaatgtgacctggttccatgccatccatgtgtctggcaccaatggcaccaagaggtttgacaaccctgtgctgccattcaatgatggagtctactttgccagcacagagaagagcaacatcatcaggggctggatttttggcaccaccctggacagcaagacccagtccctgctgattgtgaacaatgccaccaatgtggtgattaaggtgtgtgagttccagttctgtaatgacccattcctgggagtctactaccacaagaacaacaagtcctggatggagtctgagttcagggtctactcctctgccaacaactgtacctttgaatatgtgagccaaccattcctgatggacttggagggcaagcagggcaacttcaagaacctgagggagtttgtgttcaagaacattgatggctacttcaagatttacagcaaacacacaccaatcaacctggtgagggacctgccacagggcttctctgccttggaaccactggtggacctgccaattggcatcaacatcaccaggttccagaccctgctggctctgcacaggtcctacctgacacctggagactcctcctctggctggacagcaggagcagcagcctactatgtgggctacctccaaccaaggaccttcctgctgaaatacaatgagaatggcaccatcacagatgctgtggactgtgccctggacccactgtctgagaccaagtgtaccctgaaatccttcacagtggagaagggcatctaccagaccagcaacttcagggtccaaccaacagagagcattgtgaggtttccaaacatcaccaacctgtgtccatttggagaggtgttcaatgccaccaggtttgcctctgtctatgcctggaacaggaagaggattagcaactgtgtggctgactactctgtgctctacaactctgcctccttcagcaccttcaagtgttatggagtgagcccaaccaaactgaatgacctgtgtttcaccaatgtctatgctgactcctttgtgattaggggagatgaggtgagacagattgcccctggacaaacaggcaagattgctgactacaactacaaactgcctgatgacttcacaggctgtgtgattgcctggaacagcaacaacctggacagcaaggtgggaggcaactacaactacctctacagactgttcaggaagagcaacctgaaaccatttgagagggacatcagcacagagatttaccaggctggcagcacaccatgtaatggagtggagggcttcaactgttactttccactccaatcctatggcttccaaccaaccaatggagtgggctaccaaccatacagggtggtggtgctgtcctttgaactgctccatgcccctgccacagtgtgtggaccaaagaagagcaccaacctggtgaagaacaagtgtgtgaacttcaacttcaatggactgacaggcacaggagtgctgacagagagcaacaagaagttcctgccattccaacagtttggcagggacattgctgacaccacagatgctgtgagggacccacagaccttggagattctggacatcacaccatgttcctttggaggagtgtctgtgattacacctggcaccaacaccagcaaccaggtggctgtgctctaccaggatgtgaactgtactgaggtgcctgtggctatccatgctgaccaacttacaccaacctggagggtctacagcacaggcagcaatgtgttccagaccagggctggctgtctgattggagcagagcatgtgaacaactcctatgagtgtgacatcccaattggagcaggcatctgtgcctcctaccagacccagaccaacagcccaaggagggcaaggtctgtggcaagccagagcatcattgcctacacaatgagtctgggagcagagaactctgtggcttacagcaacaacagcattgccatcccaaccaacttcaccatctctgtgaccacagagattctgcctgtgagtatgaccaagacctctgtggactgtacaatgtatatctgtggagacagcacagagtgtagcaacctgctgctccaatatggctccttctgtacccaacttaacagggctctgacaggcattgctgtggaacaggacaagaacacccaggaggtgtttgcccaggtgaagcagatttacaagacacctccaatcaaggactttggaggcttcaacttcagccagattctgcctgacccaagcaagccaagcaagaggtccttcattgaggacctgctgttcaacaaggtgaccctggctgatgctggcttcatcaagcaatatggagactgtctgggagacattgctgccagggacctgatttgtgcccagaagttcaatggactgacagtgctgcctccactgctgacagatgagatgattgcccaatacacctctgccctgctggctggcaccatcacctctggctggacctttggagcaggagcagccctccaaatcccatttgctatgcagatggcttacaggttcaatggcattggagtgacccagaatgtgctctatgagaaccagaaactgattgccaaccagttcaactctgccattggcaagattcaggactccctgtccagcacagcctctgccctgggcaaactccaagatgtggtgaaccagaatgcccaggctctgaacaccctggtgaagcaactttccagcaactttggagccatctcctctgtgctgaatgacatcctgagcagactggacaaggtggaggctgaggtccagattgacagactgattacaggcagactccaatccctccaaacctatgtgacccaacaacttatcagggctgctgagattagggcatctgccaacctggctgccaccaagatgagtgagtgtgtgctgggacaaagcaagagggtggacttctgtggcaagggctaccacctgatgagttttccacagtctgcccctcatggagtggtgttcctgcatgtgacctatgtgcctgcccaggagaagaacttcaccacagcccctgccatctgccatgatggcaaggctcactttccaagggagggagtgtttgtgagcaatggcacccactggtttgtgacccagaggaacttctatgaaccacagattatcaccacagacaacacctttgtgtctggcaactgtgatgtggtgattggcattgtgaacaacacagtctatgacccactccaacctgaactggactccttcaaggaggaactggacaaatacttcaagaaccacaccagccctgatgtggacctgggagacatctctggcatcaatgcctctgtggtgaacatccagaaggagattgacagactgaatgaggtggctaagaacctgaatgagtccctgattgacctccaagaactgggcaaatatgaacaatacatcaagtggccatggtacatctggctgggcttcattgctggactgattgccattgtgatggtgaccataatgctgtgttgtatgacctcctgttgttcctgtctgaaaggctgttgttcctgtggctcctgttgtaagtttgatgaggatgactctgaacctgtgctgaaaggagtgaaactgcactacacctga 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