HIV neutralization testing with pseudovirions

University of Leipzig, Germany

Neutnet code: 1
Contact person: Christian Jassoy; christian.jassoy@medizin.uni-leipzig.de

Standard Operating Procedure

Preparation of pseudovirions (PV)

- Put 2.5 x 10^6 cells HEK293T cells with Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS) in a 25 cm^2 tissue culture flask.
- The next morning replace the medium with 4 ml fresh DMEM.
- Transfect pEnv plasmid (5 μg) together with the lentiviral expression plasmid pGJ3-luci (5 μg) that carries the firefly luciferase reporter gene as transgene under control of the spleen focus-forming virus (SFFV) promotor by the calcium phosphate method.
- Replace medium after 24 hours.
- 24 hours later, harvest cell culture supernatant that contains the pseudovirions and aliquot
- Shock-freeze the PVs using liquid nitrogen and store at –80 °C until use.
- Determine luciferase expression in cell lysates by measuring the luciferase activity at the time of pseudovirion harvest with a luminometer (Luminoscan Ascent; ThermoLabSystem) to examine transfection efficiency.

Determination of pseudovirion activity

- Use cells lines 3T3.T4.CCR5, 3T3.T4.CXCR4 (NIH AIDS Research and Reference Reagent Program) that express CD4, CCR5 or CXCR4 for the determination of pseudovirion titer and tropism. Culture cells in DMEM with 10% FBS.
- Seed cells (2 x 10^4) into a 96-well flat-bottomed tissue culture plate.
- Infect three wells of target cells with 50 µl frozen/thawed supernatant from transfected cells containing PVs pre-diluted 1:10 in DMEM without FBS.
- After 48h, quantify viral infectivity by measuring the luciferase activity. Determine mean luciferase activity from the three replicate cultures and calculate the relative vector titer/ml according to this formula:

Activity/ml = mean luciferase activity of the sample / mean luciferase activity of the negative control (cells w/o PVs) x 2 x vector dilution x 10

Neutralization test

- Optimally use culture supernatant containing 500 luciferase units of PV for each measurement. Perform individual tests with 3 replicate cultures.
- Dilute antibodies and sCD4 or sera in DMEM w/o FCS in Eppendorf tubes.
- Pipette 50 µl of each dilute in the wells of a 96 well plate.
- Add medium w/o antibodies into 6 additional wells as positive control.
- Dilute pseudovirions (PV) at a final concentration of 500 luciferase units/50µl and
- Add PVs to the antibody dilutions and medium controls.
- Incubate plates for 2 h at 37 °C, 5% CO_2.
- Dilute cells in DMEM medium with FCS
- Add 100 µl (2 x 10^4 cells) to each of the wells.
- Incubate plates for 48 h at 37 °C, 5% CO_2.

Luciferase assay

- Harvest and discard supernatant.
- Wash cells once with PBS and add 100 µl lysis buffer.
- Incubate plates for an additional 25-30 minutes on ice.
- Transfer 50 µl cell lysate to a 96 well luminometer plate.
- Add 50 µl ATP-containing assay buffer to the wells.
- Place into luminometer.
- 100 µl luciferin-containing solution is being injected automatically and luciferase acitivity is measured for 3 seconds.
- Use light units measured in the first second for calculations.

Time requirements

Manual assay with 1 antibody, 10 pseudovirions (5x96-well flat bottomed plates)

Labeling of plates	10 min.
Ab/serum titration in plastic tubes	10 min
Distribution of Ab + medium to plates, medium in control wells	20 min
Mixture of PVs in required concentrations	15 min

Distribution of PV suspension	15 min
Incubation	2 hours
Preparation of cells/counting & calibration to 2 x 10^4/well	30 min
Distribution of cells in plates	20 min

(Day 1: together with preparation time etc. approx. 6 1/2 hours)

Incubation	48 hours

Harvest of supernatant, washing with PBS	60 min (13 min/plate)
Incubation on ice	90 min (30 min/plate)
Transfer to luminometer plate	50 min (10 min/plate)
Measurement with LuminoScan	50 min (10 min/plate)

(Day 3: 4-5 hours plus 1-2 hours data management and analysis)