Neutralisation Assay

University of Oxford, United Kingdom

Neutnet code: 13

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Method

Day 0 –
Seed 96 flat bottom plates with U87.CD4.CCR5 cells at $2x10^5$ cells/ml (200ul/well), incubate overnight at 37C 5% CO2.

Day 1 –
To top well of serial dilution plate prepare starting concentrations of antibody or serum in a total of 110ul (each neutralising reagent is done in triplicate).

Take 55ul of first row and add to 55ul of media in the row 2, continue 2-fold dilution until the second to last row, leaving the last row inhibitor free.

Add 55ul of 100TCID50 of pseudovirus to each appropriate well of titrated inhibitors and incubate at 37C for 1hr. Each plate will have no virus lanes.

Remove all medium from U87 cells and add 100ul of inhibitor/pseudovirus mix, incubate overnight.

Day 2 –
Add 100ul of fresh media to all wells.

Day 4 –
Lyse cells, and test for luciferase read-out

Plate layout example

inhibitor + virus 1	inhibitor + virus 2	inhibitor + virus 3	inhibitor no virus
No inhibitor			