**2DI inhibition of H1N1 infection**

Co-infection of 2DI and H1N1 in MDCK

- seed MDCK cells on a 12 well plate

- infect cells one day after seeding, triplicate

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| --- | --- | --- | --- |
| WSN only | WSN + 2DI | Pdm09 only | Pdm09 + 2DI |
| WSN only | WSN + 2DI | Pdm09 only | Pdm09 + 2DI |
| WSN only | WSN + 2DI | Pdm09 only | Pdm09 + 2DI |

- remove the medium and wash with PBS

- infect cells with pdm09 and WSN at MOI=0.1 for 1 hour

- for the treatment group, co-infect with 2DI H7-WSN-DI597/477 at MOI=1

- remove the inoculum and wash with PBS

- add 1mL of plain MEM supplemented with 1:1000 TPCK-trpysin

- harvest supernatant at 24h post infection and store at -80ºC

Plaque assay

- seed MDCK on a 6 well plate. Each 10cm dish of MDCK can seed three 6 well plates

- 2 days after seeding, remove the medium and replace with 1mL of plain MEM

- on a 96 well plate, perform serial dilution of the virus sample

- **100** → 15uL virus sample + 135uL plain MEM → **10-1** → 15uL virus sample + 135uL plain MEM → **10-2** → 15uL virus sample + 135uL plain MEM → **10-3**...

- perform dilution up to **10-7**

- infect the cells by adding 100uL of virus from the dilution plate for one hour

- prepare the agar by mixing equal volumes of 2x MEM and 2x low melting agarose with 1:1000 TPCK-trypsin

- remove the inoculum and overlay the cells with the 3mL of the gel mixture

- allow the gel to solidify and put it back to the incubator

- 3 days after infection, fix the plate with 4%PFA, fix overnight

- after fixation, remove the PFA and the gel

- stain the cells with crystal violet

- count the plaques on the well which gives 10-100 plaques