**A549 ZIKV Infection**

Day 1 – seeding cells:

1. Seed cells in 12-well plates at density of 3x105 cells/well (1ml/well)
   1. Cells are seeded in cDMEM
   2. cDMEM recipe:
      1. 10% FBS
      2. 5ml HEPES buffer
      3. 5ml L-glut
      4. 5ml Ab/Am or Pen/Strep
      5. 5ml MEM amino acids
      6. 5ml sodium pyruvate
2. Prepare lysis buffer
   1. See calculations for lysis buffer makeup
   2. Lysis buffer recipe:
      1. RIPA buffer
      2. Okadaic acid 1:1000 of total volume
      3. Phosphatase inhibitor 1:100 of total volume
      4. Protease inhibitor 1:100 of total volume

Day 2-5 – infecting and harvesting cells:

1. Prepare virus inoculum in DMEM as shown in calculations
2. Aspirate media from wells **EXCEPT for wells labelled “cells no change”**
3. Wash cells once in 1xPBS and remove PBS wash (this is to remove serum from cells)
4. Add 200ul DMEM or virus inoculum to appropriate wells
5. Incubate plates on rocker for 2 hours at 37C
6. Remove inoculum and collect and store at -80C
7. Wash cells once with 1xPBS and remove PBS wash (this is to remove unbound virus)
8. Add 1ml cDMEM to wells
9. Return plates to incubator at 37C and allow infection to go on until appropriate time points (4h, 8h, 10h, 24h, 48h, and 72h)
10. Harvest cells in RIPA lysis buffer (50ul/well) for Western blot analysis
    1. See detailed protocol on p.4 steps 5-12 of Notebook #1 for harvesting protein lysate
11. Collect supernatant from all samples
    1. Supernatants were centrifuged at 2000rpm for 10min. at 4C
    2. Aliquot 2x 400ul of cleared supernatant into clean Eppendorf tubes and store at -80C
12. Harvest RNA lysates from cells
    1. See detailed protocol on p.14 of Notebook #1 for isolating RNA