FLUO8-AM Assay

1. Make HEPES-Hanks Buffered Solution (HHBS)

   100 mL 10X HBS
   900 mL Ultrapure water
   4.76 g HEPES
   **Sterile filter or autoclave.*

2. Make 25 mM probenecid.
   72 mg Probenecid
   0.3 mL 1 M NaOH
   9.7 mL HHBS
   *Aliquot and store at -20°C

3. Make FLuo8 stock preparation.
   Add 238.79 uL anhydrous DMSO to 1 mg Fluo8-AM (4 mM stock concentration). Aliquot in 16 uL volumes. Store at -20°C.

4. Prepare a 2X working solution in HHBS.
   16 uL Fluo8-AM (4 uM final concentration)
   32 uL 20% Pluronic F-127 (0.04%)
   320 uL 25 mM Probenecid (0.5 mM final concentration)
   Add HHBS until volume is 8 mL.

5. Prepare HHBS with 0.5 mM Probenecid
   200 uL 25 mM Probenecid
   9.8 mL HHBS

6. Add 100 uL 2x working solution to each well containing cells/media. Incubate for 1 hour (20-120 minutes recommended). Immediately before running your experiment, replace your working dye solution with HHBS with 0.5 mM Probenecid

7. Make your drug plate. Use the 96 well template. Use your 1X HHBS (with 0.5 mM probenecid)

8. Follow the steps on the machine for turning on the machine.

9. Approximately 20 minutes later, perform a yellow plate reading. First run the exposure at 0.05 without selecting calibration. Percent standard deviation will be ~5%. Next run the calibration step. Percent standard deviation should be ~1.2%. Document max, average, min value and % standard deviation.

10. Select your FLIPR template or generate it. Create a file for each day experiments are performed. It is saved in Documents → DATA→ Pierce Lab → Date

11. Run the experiment. Analyze using GraphPad Prism.

Contact information: mpierc1@midwestern.edu Updated 2/3/2020