

## Calcium imaging protocol

(Very generic. Does not describe the use of the software or the perfusion system.)

### Preparation

Set up 2 plates of cells.

Serum-starve cells 4-16 hours.

→ Remove complete media and replace with DMEM

Turn on incubator in microscope room.

Prepare 1mM Fluo-4

→ Take one 50µg tube of Fluo-4, AM, cell permeant (Life Technologies, F-14201).

Resuspend in 45µl DMSO. May be able to store unused portion at -80 when finished, but pay attention to loss of activity. Make fresh if necessary.

Prepare 20% pluronic in DMSO

→ 10mg/50µl

Mix Fluo-4 and pluronic 1:1 for working solution.

→ Assume that you need 5µl for each well (24-well format).

Prepare 10mM ionomycin in DMSO for positive control

→ Can be stored at -20° so there should be some aliquots in the freezer. If you make more, aliquot and store at -20°.

→ Should stimulate cells with ~10µM for a good response.

Prepare ligands/drugs for stimulation.

→ This is going to vary with each experiment.

### Assay

Add Fluo-4/pluronic to one well of plate #1. \*record time\*

→ Should be used at 1-5µM, so assuming 500µl of culture media, add 1-5µl. The actual amount will have to be determined empirically because it depends on cell type. Start with 2µl and adjust up (if the cells are too dim) or down (if they are too bright or don't respond well) as necessary.

Incubate for 15-60 minutes in the incubator.

→ This will also have to be determined empirically. Too short, and you don't get enough "loading". Too long, and the cells start to die. Always write down the addition time and the assay time in your lab notebook.

Remove the media from the Fluo-4/pluronic well of plate #1 and replace with 500µl DMEM.

\*record time\*

Incubate for 30 minutes.

Add Fluo-4/pluronic to one well of plate #2 and return it to the incubator. \*record time\*

Remove the media from the Fluo-4/pluronic well of plate #1 and replace with 250µl HBSS.

Place on microscope and find a good field of cells in the green channel.

Start recording.

Stimulate the cells with 250µl of the ligand/drug of interest.

→ Press the F12 key to mark addition time.

Record for 2-5 minutes.

Stimulate with ionomycin.

Record for at least one more minute.

Add Fluo-4/pluronic to the next well of plate #1 and return to incubator. \*record time\*

Remove plate #2 from incubator and repeat recording process on cells that were incubated with Fluo-4/pluronic.