Fura-2 AM Plate Reading Protocol

Obtain 2.5 x 10^5 cells per condition

Spin down cells (1100 rpm for 10 minutes if using a large tube, 30 seconds at 13.2k rpm if using small tube)

Aspirate media and try to not disturb the cell pellet

Place 1 x 10^6 cells in a 1.5 ml centrifuge tube with 1ml of experimental media + 1 ul of Fura-2 AM (use multiple tubes if over 1 x 10^6 cells are required for the experiment, mix media and fura on vortex first, then resuspend the cells in them)

Incubate the cells for 25 minutes at room temperature for the Fura-2 AM loading

Following incubation, spin the small centrifuge tube at 13.2k rpm for 30 seconds and aspirate the medium

Combine all the cells in to one 1.5ml centrifuge tube with 1 ml of Normal Ringer for washing and spin for 30 seconds at 13.2k rpm

Aspirate and resuspend in Normal Ringer at a concentration so your wanted cell amount is in 100ul of ringer

Place 100ul of cells in each well for your wanted conditions

If No Ca control is needed then with the last 100ul of cells spin + aspirate and wash with 1 ml No Ca ringer and spin + aspirate and resuspend in 100ul of No Ca ringer and place in appropriate well

Place 100ul of just Normal Ringer in a well for a blank control

Incubate the plate with the desired molecules in each condition at room temperature at this point (DMSO in control conditions)

Make up the stimulation solution of just TG at 1:9 dilution

Make sure to setup the plate reader before stimulating the cells. Using the Fura protocol 340 and 380nm wavelengths need to be recorded in a kinetic reading for the period of time that you want to observe

Add 2 ul of the stimulation solution to each condition where it is needed and place quickly in the plate reader to be read