Loading Fura-2 Protocol for HeLa Cells

1. -Dissolve Fura-2 vials in 50μl DMSO.

2. -Plate HeLa cells at varying densities from 5k to 10k, 8k usually works well

3. -Prepare labeling media: To HeLa media, add 5μl HEPES/ml and 5μl probenecid/ml.
   - For Fura-2, add 5μl dye/ml.

4. -Label: aspirate media, add 100μl labeling media to each well and incubate 45 min at 37°C.

5. -Prepare wash solution: To HBSS, add 5μl HEPES/ml and 5μl probenecid/ml.

6. -Wash: aspirate media, replace with wash solution (~100μl), aspirate, and replace with 80μl (if adding 20 μl drug for 5x dilution) wash solution (7.

7. -Prepare drug solutions: For ATP, add ~3-4 μl ATP/ml HBSS, for Ionomycin, add ~6 μl/ml HBSS (These act as positive controls for the assay)

Please note: with each cell line you will have to make adjustments since this protocol is set-up for Helas

Fura-2 AM catalog # F-1221 Molecular Probes
Probenecid catalog # P36400 Molecular Probes