Fluo-4AM Calcium Staining Protocol for ASG

 Pluronic F-127: P-3000MP (1mL)

 Fluo 4-AM: F-14201 (10 x 50ug)

 • Fluorescence intensity increase upon binding Ca2+: >100 fold
 • Kd for Ca2+ in buffer: ~335 nM
 • Exhibit fluorescence increase upon binding Ca2+ with little shift in wavelength.

1. Rinse 3x 3' **warm** **PBS** w/Ca2+ & Mg2+
2. Aspirate remaining liquid from distal bioreactor, leave liquid in recess (~4mL total will remain).
3. Stain w/1-5μM (1μM) **Fluo-4AM w/pluronic Acid**:

*•1-5mM (4mM) Fluo-4AM Stock: 50μg Fluo-4AM stock reconstituted in 11.4μL anhydrous DMSO (Use within 1 week)*

*•1-5μM (1μM): Mix 2μL of 4mM Fluo-4AM with 2μL (equal volume) pluronic acid (20% w/v) and add to 4mL PBS w/Ca2+ & Mg2+.*

1. Incubate 30' @ 37°C (22°C reduces subcellular compartmentalization)
2. Rinse 1x **fresh medium**
3. Incubate 30' @ 37°C (22°C reduces subcellular compartmentalization)
4. Rinse w/fresh medium 3'
5. Warmup bulb 30', Excitation 494 / Emission 516, 20-40x mag, full filters, full aperture
6. NIS-elements software settings:

 ≤4x4 binning, 30ms exposure, ≥2FPS, 200-500ms shutter, "Dino" profile links shutter, safely image for 10'

\*probotacid P36400\* \*antifluroscein ab\*

KCl (fw=74.5 g/mol)

Stock =2M (149 g/L)

10x Dilution = 0.2M (14.9 g/L)

 -100uL 2M stock (14.9 mg)

 -900mL PBS++

PBS = 2.7 mM (200 mg/L)

Make cultures in PBS 5 mM (372 mg/L)

 -Add 172.5 mg/L

 - For 1 mL culture, add 172 ug KCl

 - 11.5 uL 0.2M KCl

Make cultures in PBS 10 mM (745 mg/L)

 -Add 545 mg/L

 -For 1 mL culture, add 545 ug KCl

 -36 uL 0.2M KCl