Cell Biology and Bioimaging Core Staining Protocols

BODIPY 493/503: Our stock is 19mM in DMSO (-30°C freezer). Working solutions range around 10-80 µM but you should experiment to determine the best staining for your sample. Ex/Em = 493/503.

BODIPY 558/568 C_{12}: Stock aliquots in DMSO (-30°C freezer) at 2.12 mM. Working solutions range around 10-80 uM but you should experiment to determine the best staining for your sample. Ex/Em = approx 558/568; emits into red region.

BODIPY FL C5-ceramide complexed to BSA: Reconstitute 5 mg unit in 150 µM sterile distilled water. This stock is 0.5 mM BODIPY FL ceramide, 0.5 mM BSA. Working dilution is 100X (5 µM ceramide). Incubate for 30 minutes at 4°C, wash with ice-cold medium 2X and incubate in fresh medium at 37°C for a further 30 minutes. Wash again in fresh medium before imaging. Ex/Em = 505/511 and 620 nm.

Calcein, AM: Stock solution is 1mM in DMSO. Recommended working dilution is 10000X (100 nM). Incubate at 37°C for 15-30 min, wash cells and (optional) incubate for an additional 15 min to allow esterase activation to go to completion. Ex/Em = 488/530 nm.

CellTracker Blue CMHC: Stock solution is 10mM in DMSO. Working dilution is 20,000X (.5 µM) to 200X (50) in serum free medium. Incubate for 15-45 minutes at 37°C, replace with fresh media and incubate for 30 minutes at 37°C. NOTE: Independent observations have noticed a potential cytotoxic effect with this dye at concentrations above 10 µm (CHOK1 cells). Wash with PBS before imaging or fixing. Ex/Em = 372/470 nm.

CellTracker Green CMFDA: Same as CellTracker Blue. No cytotoxic effects were seen at concentrations of 25 and 10 µM with CHOK1 cells. Ex/Em = 492/517 nm.

DAPI: Stock solution is 10.9 mM in dH_2O. Dilute 5 µL stock in 995 µL PBS (Solution 1). This is stable in the dark at 4°C for at least one month. Add 10 µL Solution 1 to 990 µL PBS to create the working solution (0.5 µg/mL or 1.09 µM). Incubate for 5-15 minutes then wash 2X with PBS. Ex/Em = 360/460 nm.

Dihydroethidium: Ex/Em of oxidized form (EBr) = ~488/~605 nm.

DRAQ5: Stock solution is 5mM in dilute acid. Working solution is usually added directly to growth medium at 1-20µM and allowed to incubate for about 5 minutes before imaging. Note: There is some excitation with 488nm so this can be used instead of the 633 line. Ex/Em = 647/670 nm.

ER-Tracker Green (glibenclamide BODIPY FL): Same as ER-Tracker Red. Ex/Em = 504/511.

ER-Tracker Red (BODIPY TR glibenclamide): Stock solution is 1 mM in DMSO. Working concentration is around 1 µM however you should experiment with lower concentrations to

minimize potential labeling artifacts. Stain cells at 37°C in HBSS/Ca/Mg for 15-30 min. Replace staining medium with fresh medium and image. Fix cells using 4% formaldehyde for 2 min at 37°C, wash 2X with buffer, mount, and image. Permeabliization with Triton X-100 will release dye so DON'T PERMEABILIZE. Ex/Em = 587/615. 

FM 4-64FX Membrane Stain: Provided in tubes of 100µg. Working solution is 5 µg/mL dye in ICE-COLD HBSS; keep the staining solution cold if staining living cells as the dye will quickly be internalized. Quickly immerse cells/tissue in staining solution, ON ICE, for 1 min. Mount on a slide, seal, keep on ice, image immediately without washing. Alternatively, you can fix the specimen after staining with ICE-COLD 4% formaldehyde in HBSS ON ICE for 10 min. Rinse 3X with HBSS, mount and image. Ex/Em = 565/744. 

Fura-2 AM: Stock solution is 1mM in DMSO. Mix stock solution 1:1 with 20% Pluronic F-127 in DMSO. Add 2-10 µL of this mixture per mL of serum-free medium. This gives 1-5 µM fura-2 AM, 0.02-0.1% Pluronic F-127 and 0.2-1.0% DMSO. Incubate at 37°C for 15-30 minutes, wash cells twice and (optional) incubate for a further 15-30 minutes to allow esterase activation to go to completion. Ex/Em = 340 and 380/510 nm (ratio). 

Fluo-4 AM: Same as fura-2 AM. Ex/Em = 488/530 nm. 

Hoechst: Current stock is 10mM in PBS. Incubate cells or tissue with anywhere from 1 ug/ml to 10 ug/ml – about 1:1000 dilution is a good starting point. 

JC-1: Stock solution is 5mg/ml (7.7 mM) in DMSO. Working dilution 500X (15 µM) to 5000X (1.5 µM). Incubate cells for 10 minutes at 37°C. Wash in fresh media. Ex/Em = 488/530 and 590 nm.  

Lectin from Bandeiraea simplicifolia – FITC conjugate (Sigma L2895): Comes as a lyophilized powder. Resuspend in PBS and use at a concentration from 10ug/ml to 40ug/ml. 

LysoTracker Red: Stock solution is 1mM in DMSO. Suggested working dilution is 10,000X (100 nM). Incubate for 30 minutes to 2 hours at 37°C then wash with fresh medium or buffer solution. Ex/Em = 568/590 nm. 

LysoTracker Yellow-HCK-123: Same as LysoTracker Red. Ex/Em = 465/535. 

MitoTracker Green FM: Stock solution is 1 mM in DMSO. Suggested working dilution is approximately 10,000X (100 nM). Incubate for 15-45 minutes at 37°C, remove media and replace with fresh before imaging. Ex/Em = 488/530 nm. 

MitoTracker Red CMXRos : Dissolve one 50 µg vial in 470.35 µL DMSO (200 µM stock). Suggested working range varies from 500 nM to 10 nM (400X – 20000X). Dilute stock to working concentration in culture media or buffer of choice, prewarm, add to cells and incubate 15-45 minutes at 37°C, remove media and replace with fresh before imaging or fix. Ex/Em = 568/600 nm. 

MitoTracker Deep Red 633 FM: Sames as MitoTracker Red. Ex/Em = 644/665. 

Oregon Green 488 BAPTA-2 AM: Stock in DMSO (2-5 mM), working solution 1-10 uM in buffer of choice. Incubate cells with OG for 20 min to 1 h at RT or 4C. Wash cells and image. Ex/Em = 494/523 nm. 

Propidium Iodide (PI): Stock solution is 1 mM in dH_2O. Working dilution is 100X (10 µM) to 1000X (1µM). Ex/Em = 488/620 nm. 

Sulforhodamine 101 

SYTO 60 (and other reds): Stock solution is 5mM in DMSO. Working dilution is 1000X (5 µM) to 100,000X (50 nM). Incubate 10-120 minutes before imaging. Ex/Em = 633/670 nm. 

TO-PRO-3: Stock solution is 1mM in DMSO. Working dilution is 1000X (1µM) to 10000X (0.1µM). Ex/Em = 633/670 nm. 

TubulinTracker Green: FOR LIVE CELL IMAGING ONLY! Stock solution is 1mM in DMSO. Each tube of 1 mM stock solution should be enough for about 280 assays (96 well plate format) using 1 mL per assay at a final concentration of 250 nM. Vortex Component B vial and add an equal volume of 20% Pluronic F-127 in DMSO to a thawed aliquot of 1mM TubulinTracker Green stock to make a 500 µM intermediate stock solution. This intermediate stock soln. is stable for at least 2 weeks when stored at ≤ 20°C. Vortex then dilute the 500 µM intermediate stock solution in HBSS/Ca/Mg at a concentration of 50 – 250 nM. Stain with only freshly made solution at 37°C. Incubate for 30 min at 37°C. Ex/Em = 494/522 nm. 

Wheat Germ Agglutinin conjugates: Stock solution is 1mg/ml in PBS. Working dilution is 10X (100 µg/ml) to 1000X (1 µg/ml). Incubate for 10-30 minutes at room temperature then wash with fresh buffer. Plasma membrane-specific labeling works best if the working dilution is made in HBSS. WGA-Alexa 350 Ex/Em = 346/442, WGA-Oregon Green 488 Ex/Em = 496/524, WGA-tetramethylrhodamine Ex/Em = 555/580, WGA-Texas Red-X Ex/Em = 595/615.