Cell Biology and Physiology Lab (Capers)

Fluorescence Microscopy – Protocol for Invitrogen Mitotracker Green FM

MitoTracker® dyes are cell permeable probes that contain a mildly thiol-reactive chloromethyl moiety for mitochondrial labeling. To label mitochondria, cells are simply incubated with MitoTracker® probes, which passively diffuse across the plasma membrane and accumulate in active mitochondria.

Procedure:

1. Each vial of MitoTracker® Green FM (kept frozen) contains 50 µg lyophilized solid.
   * To make a 1 mM stock solution, reconstitute the solid in 74.4 µl high quality DMSO.
2. Make a 100-400nM solution in media (10ml per flask of cells)
   * C1V1 = C2V2
   * Convert 400nM to mM = 0.0004mM
   * 1mM (x) = 0.0004mM (5ml) gives you 0.002ml or 2µl per 5ml media
3. Aspirate old media from cells
4. Add media containing probe
5. Incubate between 15-30 min at 37ºC. After incubation, the cells must be imaged live; DO NOT FIX!

