MitoTracker staining for C2C12 myotubes

Procedure

1. Treat cells as desired in 35-mm dish with 3 ml growth medium.
2. Dilute MitoTracker with the growth medium at 1:2000 and pre-warm it at 37°C.
3. Aspirate medium and add 1 ml diluted MitoTracker and incubate for 30' at 37°C.
4. Dilute DAPI in PBS by adding 1 µl DAPI stock (7.15 mM) to 23.8 ml of PBS and pre-warm it at 37°C.
5. Aspirate the MitoTracker medium and add DAPI solution and incubate for 1'.
6. Wash the cells with pre-warmed growth medium 2' x 2.
7. Prepare medium containing 3.7% formaldehyde and pre-warm it at 37°C.
8. Incubate the myotubes in 3.7% formaldehyde at 37°C for 15'.
9. Rinse the cells in PBS 5' x 2.
10. Mount the coverslips with VectorSheild.

Reconstitute MitoTracker (Tracker Red CMXRos, Molecular Probes Cat# M7512, 50 µg) in 94.1 µl of DMSO (1 mM), and store it in 10 µl aliquots at -20°C in Box 71.
11.