Mitochondria Labelling - MitoTracker™ Protocol

Reference

1. Afreen, S., Riherd Methner, D. N., & Ferreira, A. (2017). Tau_{45-230} association with the cytoskeleton and membrane-bound organelles: Functional implications in neurodegeneration. *Neuroscience*, *362*, 104–117. https://doi.org/10.1016/j.neuroscience.2017.08.026

Kit

1. Invitrogen™ MitoTracker™ Red CMXRos, Catalog# M7512

Solution

1. Neuronal maintenance medium (N2): 1X MEM (Invitrogen), 0.6% D-glucose, 0.1% ovalbumin, 1 mM sodium pyruvate, 5 µg/ml insulin, 20 nM progesterone, 100 µM putrescine, 30 nM selenium dioxide, 100 µg/ml apo-transferrin. Filter-sterilize and store at 4°C. Keep component stock solutions at -20°C.

Protocol

1. Reconstitute 1 vial of MitoTracker™ in DMSO to 1 mM, this is the stock solution.
2. Dilute enough stock in N2 medium to a final working solution concentration of 100 µM.
3. Add enough working solution of MitoTracker™ to dishes to give a final concentration of 400 mM, adjust dish medium accordingly.
4. Incubate for 1hr at 37°C.
5. Wash cells once with N2 medium.
6. Observations of axonal transport should be performed at least 3 hr after washout of organelle probes in glia-conditioned N2 medium without phenol red in order to reduce fluorescent background.