



Aug 20, 2025

MitoTracker staining of fibroblasts

DOI

<https://dx.doi.org/10.17504/protocols.io.j8nlkyoo6g5r/v1>

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Protocol Citation: Ashley Seifert, Ebenezer Aryee 2025. MitoTracker staining of fibroblasts. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.j8nlkyoo6g5r/v1>

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Protocol status: Working

We use this protocol and it's working

Created: August 16, 2025



Last Modified: August 20, 2025

Protocol Integer ID: 224797

Keywords: ASAPCRN, fibroblast, mitochondria, mitotracker, mitochondria in fibroblast, visualizing mitochondria, fibroblasts this protocol, fibroblast, seifert lab

Funders Acknowledgements:

Aligning Science Across Parkinson's

Grant ID: ASAP-020495

NIH

Grant ID: R01AR070313

Abstract

This protocol describes the procedure for visualizing mitochondria in fibroblasts with MitoTracker used in the Seifert Lab.

Protocol materials

☒ DMEM and Hams F-12 50/50 **Fisher Scientific Catalog #MT10090CV**

☒ FBS (HyClone)

☒ Antibiotic/Antimycotic (100X) **Thermo Fisher Scientific Catalog #15240062**

☒ Tissue culture dish (10 cm) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #Z707651**

☒ HBSS **Thermo Fisher Scientific**

☒ MitoTracker® Red CMXRos **Cell Signaling Technology Catalog #9082**

☒ 1X PBS

☒ 4% Formaldehyde, Methanol-Free **Cell Signaling Technology Catalog #47746**

☒ Acetone **Fisher Scientific Catalog #A18P-4**

☒ DAPI (4,6-diamidino-2-phenylindole, dihydrochloride) **Thermo Fisher Scientific Catalog #D1306**

☒ HBSS **Gibco - Thermo Fisher Scientific Catalog #14170-112**

☒ Sterile deionized H₂O

☒ Prolong Gold Antifade Mountant **Thermo Fisher Scientific Catalog #P36930**

☒ MitoTracker Green FM **Cell Signaling Technology Catalog #9047S**



- 1 Seed fibroblasts in 24 well plates on cover slips at 5,000 cells per well
- 2 Culture cells in
⊗ DMEM and Hams F-12 50/50 **Fisher Scientific Catalog #MT10090CV**
supplemented with 10% ⊗ FBS (HyClone) and 1%
⊗ Antibiotic/Antimycotic (100X) **Thermo Fisher Scientific Catalog #15240062** at
🌡 37 °C under 3% O₂ and 5% CO₂ in
⊗ Tissue culture dish (10 cm) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #Z707651**
for ⌚ 48:00:00
- 3 Remove media
- 4 Wash cells with warm ⊗ HBSS **Thermo Fisher Scientific**

MitoTracker Red

- 5 Follow this procedure for MitoTracker Red staining and fixed cell imaging
 - 5.1 Add [M] 0.3 µL/mL
⊗ MitoTracker® Red CMXRos **Cell Signaling Technology Catalog #9082** in FBS-free DMEM for ⌚ 00:30:00
 - 5.2 Wash cells twice with ⊗ 1X PBS
 - 5.3 Fix for ⌚ 00:15:00 in the dark with warm
⊗ 4% Formaldehyde, Methanol-Free **Cell Signaling Technology Catalog #47746**
 - 5.4 Remove formaldehyde and wash cells twice with ⊗ 1X PBS for ⌚ 00:05:00



5.5 Add  500 μ L chilled  Acetone **Fisher Scientific Catalog #A18P-4** to each well and incubate for  00:05:00

5.6 Remove acetone and wash cells once with  1X PBS

5.7 Add  DAPI (4,6-diamidino-2-phenylindole, dihydrochloride) **Thermo Fisher Scientific Catalog #D1306** diluted 1:2,000 in  HBSS **Gibco - Thermo Fisher Scientific Catalog #14170-112** to each well and incubate for  00:15:00

5.8 Remove DAPI solution and wash cells with  Sterile deionized H₂O

5.9 Air dry coverslips

5.10 Mount on glass slides with  Prolong Gold Antifade Mountant **Thermo Fisher Scientific Catalog #P36930**

MitoTracker Green

6 Follow this procedure for MitoTracker Green staining and live cell imaging

6.1 Prepare mix by adding  2 μ L of  MitoTracker Green FM **Cell Signaling Technology Catalog #9047S** to  12 mL and warming

6.2 Incubate cells in staining mix in FBS-free DMEM for  00:30:00

6.3 Wash cells once with  HBSS **Gibco - Thermo Fisher Scientific Catalog #14170-112**



- 6.4 Live Image immediately. Cells should be cultured in **Cellvis 24 Well glass bottom plate with high performance #1.5 cover glass # NC0397150** for optimal imaging.