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Working

UC Davis - Immunohistochemistry Mitochondrial marker [↗](#)

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[dx.doi.org/10.17504/protocols.io.y86fzze](https://doi.org/10.17504/protocols.io.y86fzze)

Mouse Metabolic Phenotyping Centers
Tech. support email: info@mmpc.org

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ABSTRACT

Summary:

Mitochondria are the power house of the cell. They are distinct organelles with two membranes. Usually they are rod shaped, however they can be round. Several diseases and disorders including schizophrenia, bipolar disorder, dementia, Alzheimer's disease, Parkinson's disease, epilepsy, stroke, cardiovascular disease, retinitis pigmentosa, and diabetes mellitus have all shown association to mitochondrial dysfunction and disorder.

EXTERNAL LINK

<https://mmpc.org/shared/document.aspx?id=258&docType=Protocol>

MATERIALS

NAME	CATALOG #	VENDOR
Paraformaldehyde	P6148	Sigma Aldrich
BSA	30-AB79	Fitzgerald
Saponin	47036	Fluka
Prolong gold (with dapi)	P36930 (P36931)	Life Technologies
Xylene		
Ethanol		
Tri-sodium citrate		
anti-mitochondria Ab [MTC02]	ab3298	Abcam
0.1M Phosphate Buffered Saline pH 7.4		
coverslip	2935-245	Corning
goat anti-mouse IgG Alexa 555	A-21424	Molecular Probes

MATERIALS TEXT

Reagent Preparation:

Reagent1: Block/Perm

Reagents and Materials:

BSA

Saponin

0.1M Phosphate buffered saline (PBS), pH 7.4

Procedure:

For 100mL combine 1g BSA and 0.05g Saponin in 100mL PBS

Reagent 2: 4% Paraformaldehyde

Reagents and Materials:

Paraformaldehyde

0.1M Phosphate buffered saline (PBS), pH 7.4

Procedure:

For 100mL combine 4g Paraformaldehyde 95mL PBS. In a fume hood, heat solution to 55°C on a heat plate while stirring. Add 2-3 drops of concentrated HCl to bring into solution. Cool to room temperature and adjust pH to 7.4. Bring up to 100 mL and then sterile filter with 0.22 µM filter.

Reagent 3: Sodium Citrate Buffer (10mM Sodium Citrate, 0.05% Tween 20, pH 6.0)

Reagents and Materials:

Tri-sodium citrate (dihydrate) 2.94 g

Distilled water 1000 ml

Procedure:

Mix to dissolve. Adjust pH to 6.0 with 1N HCl and then add 0.5 ml of Tween 20 and mix well. Store this solution at room temperature for 3 months or at 4 C for longer storage.

Note:

Sigma-Aldrich [RRID:SCR_008988](#)

Life Technologies, [RRID:SCR_008817](#)

Abcam, [RRID:SCR_012931](#)

Fitzgerald Industries International [RRID:SCR_003237](#)

anti-mitochondria Ab [MTC02] #ab3298, Cite this (Abcam Cat# ab3298, [RRID:AB_303683](#))

goat anti-mouse IgG Alexa 555 #A-21424, Cite this (Thermo Fisher Scientific Cat# A-21424, [RRID:AB_141780](#))

SAFETY WARNINGS

WARNING:

Paraformaldehyde is, toxic, flammable and considered a carcinogen and methanol are all flammable and should be used in fume hood away from open flames or sparks

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions established by CDC when handling and disposing of infectious agents.

BEFORE STARTING

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1 Frozen tissue sections (10µM) on slides (tissues embedded in OCT) are then fixed in 4% paraformaldehyde for 5 minutes.

- a. Alternatively paraffin embedded sections are deparaffinized through 3 100% xylene 3 min wash
 - b. Sections were rehydrated through 100%, 95%, 70%, 50%, and 30% then into deionized water (3min each).
 - c. Antigens are unmasked by steaming in Sodium citrate for 10 min.
- 2 Wash three times for ten minute in PBS
 - 3 Blocked and permeablize with Block/Perm Solution for 30 minutes.
 - 4 Incubate with anti-mitochondria antibody [MTC02] diluted 1:500 in Block/Perm overnight at 4°C.
 - 5 Wash three times in Block/Perm for 10 minutes
 - 6 Incubated in goat anti-mouse IgG Alexa 555 (1:750) for 1 hour at room temperature.
 - 7 Washing three times in Block/Perm and briefly in PBS
 - 8 Fixed with 4% Paraformaldehyde for 5 minutes.
 - 9 Washed with PBS three times for 10 minutes each wash
 - 10 Coverslip mounted with Prolong gold antifade reagent.



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