



MMPC Immunohistochemistry

BODIPY

Version: 1
 Modified from:
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Summary: BODIPY® 493/503 can be used as a stain for neutral lipids and as a tracer for oil and other nonpolar lipids. It has uses for identifying and quantifying lipid droplets in various cell and tissue type. Abnormal lipid droplet accumulation can be associated with various metabolic disorders such as Diabetes, cardiovascular disease and cognitive impairment.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Paraformaldehyde	sigma	P6148
Prolong gold (with dapi)	Life technologies	P36930 (P36931)
Xylene		
Ethanol		
Tri-sodium citrate		
BODIPY® 492/515	Life Technologies	D3922
0.1M Phosphate Buffered Saline pH 7.4		
coverslip	Corning	2935-245

Protocol:

WARNING:

Paraformaldehyde is, toxic, flammable and considered a carcinogen

Xylene, ethanol 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.

1. Frozen tissue sections (10 μ M) 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. The sections were then incubated in 0.1mg/ml BODIPY® 492/515 in DMSO for 30 minutes in the dark.
4. Wash three times for ten minute in PBS
5. Coverslip mounted with Prolong gold antifade reagent with 4',6-diamidino-2-phenylindole (DAPI).

Reagent Preparation:

Reagent 1: 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 μ M 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.