

JAN 22, 2024

Immunostaining

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ASAP Collaborative Research Network



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ABSTRACT

This is a protocol that describes how to use antibody staining to detect cellular epitopes by immunofluorescence microscopy.

ATTACHMENTS

260-2491.docx





dx.doi.org/10.17504/protocols.io. e6nvwd7n7lmk/v1

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https://dx.doi.org/10.17504/protoc ols.io.e6nvwd7n7lmk/v1

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Protocol status: Working We use this protocol and it's working

Created: Jan 16, 2024



Last Modified: Jan 22, 2024

MATERIALS

PROTOCOL integer ID: 93738

Cell culture

Funders Acknowledgement:

Aligning Science Across Parkinson's Grant ID: ASAP-000282 25-50% confluent wells of cells growing on coverslips in 24-well plates.

Equipment

- Platform shaker
- Microcentrifuge

Buffers & Reagents

4% paraformaldehyde (diluted from

Pierce™ 16% Formaldehyde (w/v), Methanol-free **Thermo**Fisher Catalog #28908

) in 1X PBS (diluted from

PBS (10X), pH 7.4 Thermo Fisher Scientific Catalog #70011051

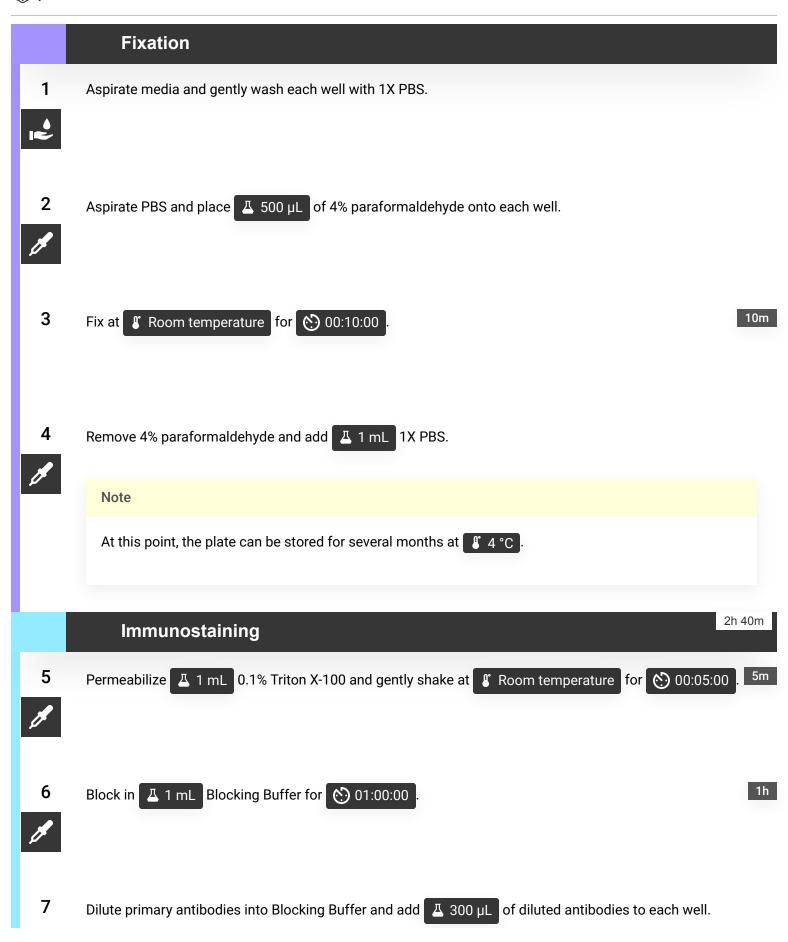
- 1X PBS
- 0.1 % Triton X-100 in PBS
- Blocking Buffer:

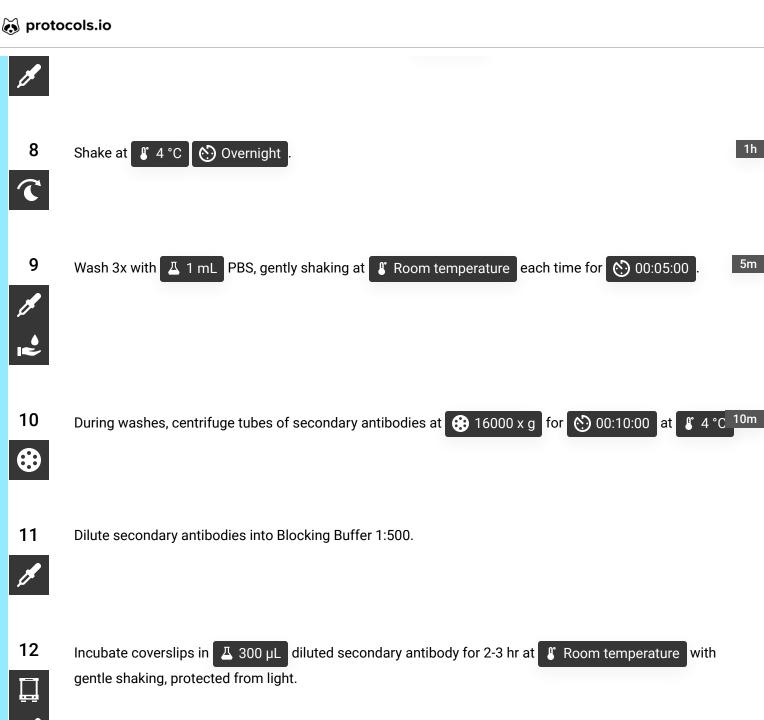
A	В
Milk powder (Sucofin)	5%
Triton X-100	0.1%
PBS	pH 7.4

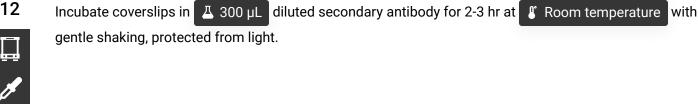
2 drops/ml

NucBlue™ Fixed Cell ReadyProbes™ Reagent **Thermo**Fisher Catalog #R37606

- Fluorescence Mounting Medium Agilent Technologies Catalog #S302380-2
- Appropriate primary and fluorophore-conjugated secondary antibodies
- Microscope slides





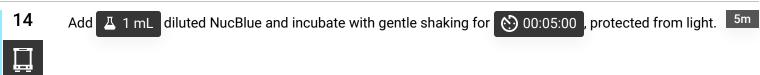


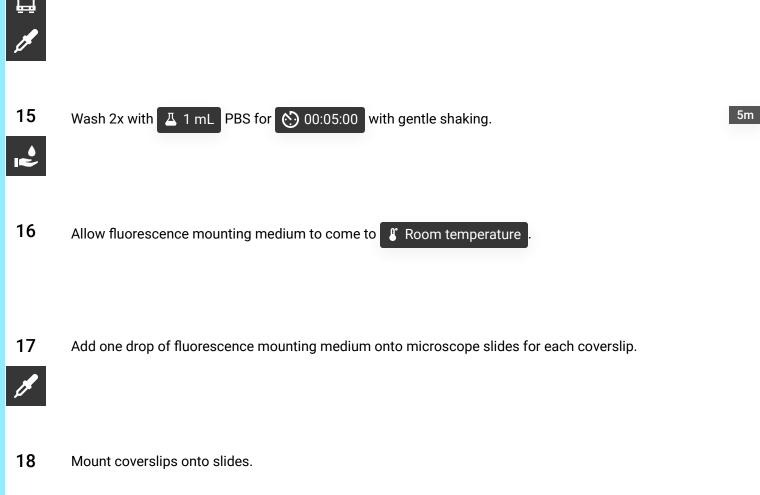
13 Wash with A 1 mL PBS for 00:05:00 with gentle shaking.

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5m

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Let slides cure at Room temperature Overnight, protected from light.

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