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

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Immunostaining

Cole S Sitron[1], Victoria A Trinkaus[1], F Ulrich Hartl[1]

[1]Department of Cellular Biochemistry, Max Planck Institute of Biochemistry

ASAP Collaborative Research Network

C Cole Sitron

ABSTRACT

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

ATTACHMENTS

260-2491.docx

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MATERIALS

Cell culture

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	B
	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

Fixation

1 Aspirate media and gently wash each well with 1X PBS.

2 Aspirate PBS and place 500 µL of 4% paraformaldehyde onto each well.

3 Fix at Room temperature for 00:10:00. 10m

4 Remove 4% paraformaldehyde and add 1 mL 1X PBS.

> **Note**
>
> At this point, the plate can be stored for several months at 4 °C.

Immunostaining 2h 40m

5 Permeabilize 1 mL 0.1% Triton X-100 and gently shake at Room temperature for 00:05:00. 5m

6 Block in 1 mL Blocking Buffer for 01:00:00. 1h

7 Dilute primary antibodies into Blocking Buffer and add 300 µL of diluted antibodies to each well.

8 Shake at 4 °C Overnight. 1h

9 Wash 3x with 1 mL PBS, gently shaking at Room temperature each time for 00:05:00. 5m

10 During washes, centrifuge tubes of secondary antibodies at 16000 x g for 00:10:00 at 4 °C 10m

11 Dilute secondary antibodies into Blocking Buffer 1:500.

12 Incubate coverslips in 300 µL diluted secondary antibody for 2-3 hr at Room temperature with gentle shaking, protected from light.

13 Wash with 1 mL PBS for 00:05:00 with gentle shaking. 5m

14 Add 1 mL diluted NucBlue and incubate with gentle shaking for 00:05:00, protected from light. 5m

15 Wash 2x with 1 mL PBS for 00:05:00 with gentle shaking. 5m

16 Allow fluorescence mounting medium to come to Room temperature.

17 Add one drop of fluorescence mounting medium onto microscope slides for each coverslip.

18 Mount coverslips onto slides.

19 Let slides cure at Room temperature Overnight, protected from light. 5m