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Immunostaining

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

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

C Cole Sitron
Max Planck Institute of Biochemistry

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We use this protocol and it's working

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Abstract

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

Attachments

DOCX

260-2491.docx

16KB

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

Troubleshooting

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