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Immunocytochemistry

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ABSTRACT

Immunocytochemistry is a technique that uses antibodies to detect antigens in cells. Here we describe the basic steps for fixing and labeling cells in culture with a primary antibody against a target protein and a fluorescent secondary antibody. This protocol outlines the steps for fixing and labeling HeLa cells for a target protein using the formaldehyde fixation method. The protocol may need to be optimized for different cells, target proteins, etc.

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GUIDELINES

Workflow Timeline:
Day 1: Seed cells
Day 3-4: Fix and label cells

Tips and Troubleshooting:
We recommend wiping down all pipettes and equipment with 10% bleach prior to use.

The optimal fixation method will vary depending on the sample type and the protein of interest. You may need to try a variety of fixation methods to find the best conditions for your target.

The optimal antibody concentration will vary between antibodies. Review the manufacturer's instructions before starting your experiment and consider titrating your antibody to determine the optimal dose.

To ensure that your antibody is both functioning as expected and specific, include a positive control sample that you know expresses the protein, such as cells transfected with a plasmid to express the protein of interest, and a negative control sample such as cells that do not express the protein of interest.

MATERIALS TEXT

Equipment:

- Pipette controller
- Pipette tips and pipettes
- Rocking platform
- Tweezers
- Fluorescent microscope
- 0.5–10 µL single channel pipette
- 2–20 µL single channel pipette
- 20–200 µL single channel pipette
- 200–1000 µL single channel pipette

Reagents and Consumables:

- 1X PBS
- Microcentrifuge tubes
- Sterile Poly-D-lysine coated coverslips
- HeLa cells
- 24-well plate
- 4% Paraformaldehyde
- 5 mg/mL 4',6-diamidino-2-phenylindole (DAPI)
- Bovine serum albumin (BSA)
- Triton X-100
- Primary antibody
- Secondary antibody
- Deionized water
- Microscope slide
- Anti-fade mounting medium
- Laboratory wipes
- 15 mL conical tubes
- 50 mL conical tubes

Reagent preparation:

Permeabilization buffer:
Dilute 20 µL of Triton X-100 in 10 mL PBS.

Blocking buffer:
Dilute 0.5 g BSA and 30 µL Triton X-100 in 10 mL PBS.

Antibody dilution buffer:
Dilute 0.5 g BSA and 150 µL Triton X-100 in 50 mL PBS.

300 nM DAPI working solution:
Prepare a 300 µM DAPI stock solution by diluting 2.1 µL of the 5 mg/mL DAPI solution to 100 µL PBS. Protect from light.
Prepare a 300 nM DAPI working solution by diluting 5 µL of the 300 µM DAPI stock solution into 5 mL PBS. Protect from light.

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BEFORE STARTING

See the Materials section for preparation of necessary stock solutions.

Refer to the manufacturer's instructions for additional information specific to your antibodies, such as antibody concentrations, incubation times, and recommended compatible reagents.

Secondary antibodies must match the host species of the primary antibody. For example, use an anti-mouse secondary antibody for primary antibodies raised in a mouse.

Seeding cells

1 Place a sterile poly-D-lysine coated coverslip in each well of a 24-well cell culture treated plate.

2 Seed 5*10^3 HeLa cells per well.

3 Allow the HeLa cells to grow to the desired density before labeling.

Fixing and permeabilizing cells

4 Gently aspirate the media from the 24-well plate.

5 Wash each well with 500 µL of PBS, remove the wash, and dispose of it in an appropriate waste container.

6 Fix each well with 500 µL of cold 4% paraformaldehyde in PBS on ice for 00:15:00 . 15m

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Note

While 4% Paraformaldehyde fixation works well for many target proteins, it may not be the best fixation method for all. Alternative fixation methods such as methanol or acetone may be better for some applications.

7 Remove the paraformaldehyde and follow your institution's laboratory safety guidelines for disposing of waste in the appropriate container.

8 Wash 3x for 00:05:00 in 500 µL PBS on a rocking platform. 5m

9 Permeabilize cells for 00:10:00 at Room temperature on a rocking platform in 500 µL permeabilization buffer. 10m

10 Remove the permeabilization buffer and dispose of it in an appropriate waste container.

11 Wash 3x for 00:05:00 in 500 µL PBS on a rocking platform. 5m

Labeling with antibody

3h 15m

12 Block for 00:20:00 at Room temperature on a rocking platform in 500 µL blocking buffer. 20m

13 Remove the blocking buffer and dispose of it in an appropriate waste container.

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14 Dilute the primary antibody to the desired concentration in antibody dilution buffer.

> Note
>
> The optimal antibody concentration will vary but generally ranges from 1-10 µg/mL.

15 Add 500 µL of the diluted antibody to the wells and incubate 02:00:00 at Room temperature. 2h

16 Remove the primary antibody and dispose of it in an appropriate waste container.

17 Wash 3x for 00:05:00 in 500 µL PBS on a rocking platform. 5m

18 Dilute the fluorescently-labeled secondary antibody to the desired concentration in antibody dilution buffer.

> Note
>
> The optimal antibody concentration will vary but generally ranges from 1-10 µg/mL.

19 Add 500 µL fluorescently-labeled secondary antibody to the wells and incubate 00:30:00 at Room temperature in the dark. 30m

> Note
>
> The plate can be wrapped in foil to block light.

20 Remove the secondary antibody and dispose of it in an appropriate waste container.

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21 Wash 3x for 00:05:00 in 500 µL PBS on a rocking platform. 5m

22 (Optional) Counterstain nuclei with 500 µL of 300 nM DAPI working solution for 00:10:00 at Room temperature in the dark. 10m

23 Remove the DAPI and dispose of it in an appropriate waste container.

24 Wash 3x for 00:05:00 in 500 µL PBS on a rocking platform. 5m

25 Use tweezers to gently remove the coverslip.

26 Blot the coverslip with a laboratory wipe to remove excess liquid.

27 Add 1 drop of anti-fade mounting medium to the microscope slide.

28 Gently place the coverslip on the microscope slide with the cell side facing down.

29 Observe the cell labeling on a microscope with appropriate fluorescent filters.

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