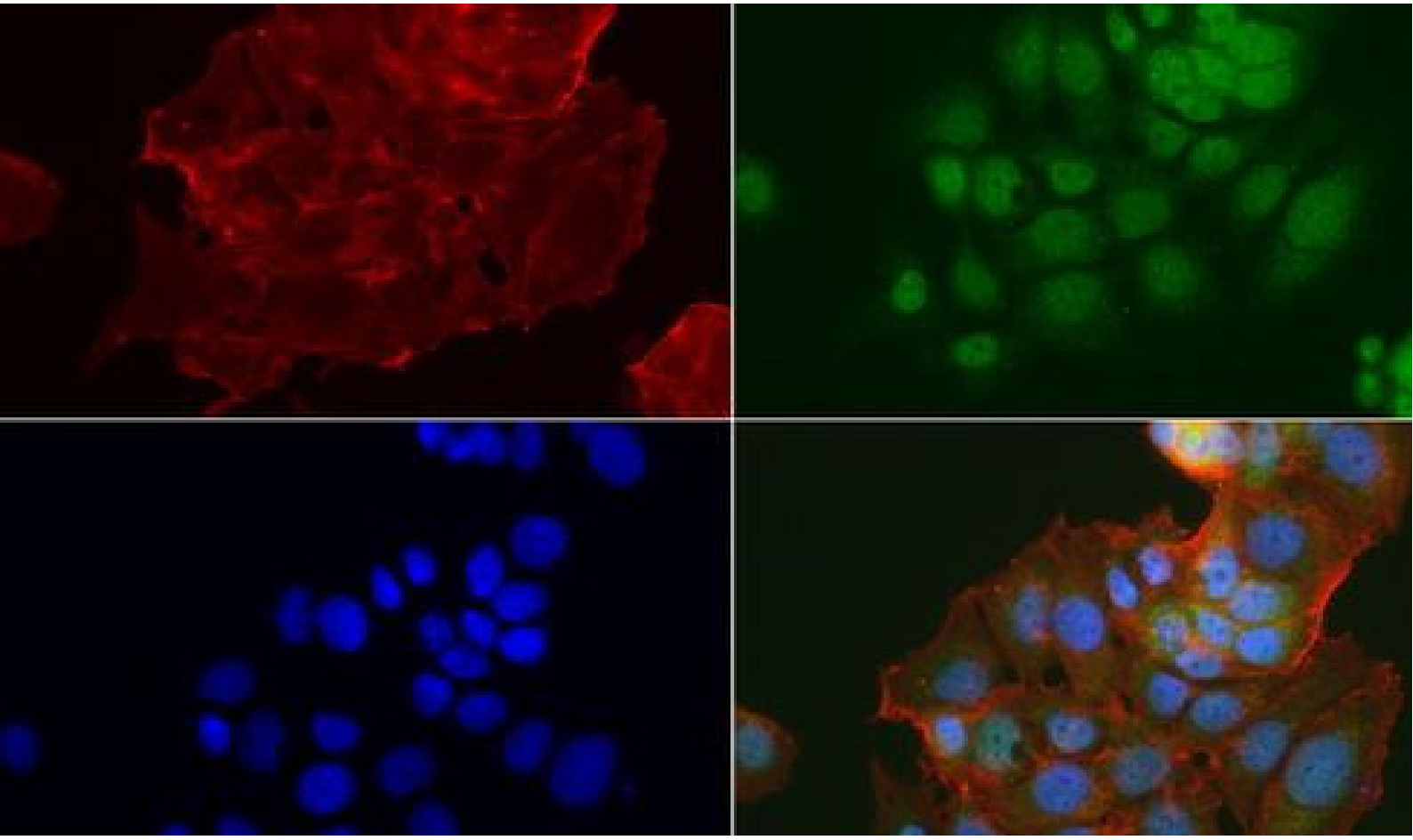
Immunofluorescent Staining Protocol

Immunocytochemistry



Solutions and Reagents

Fixation permeabilization

Staining

Protocol Overview

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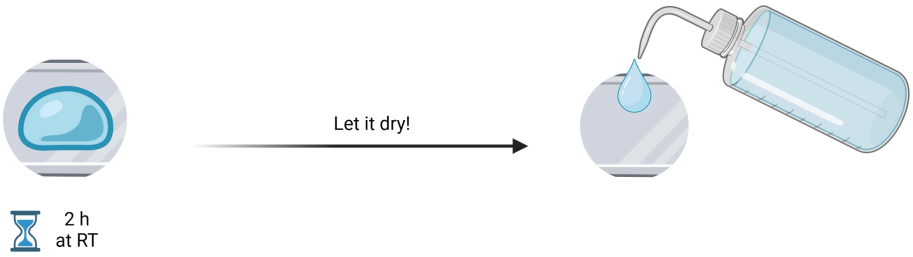
Solutions and Reagents

- 1X Phosphate Buffered Saline (PBS): Dissolve 8g NaCl, 0.2g KCl, 1.15g Na_2HPO_4 and 0.2g KH_2PO_4 in 800mL distilled water (dH_2O). Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at room temperature
- Poly-L-lysine solution: 0.1mg/ml in 1xPBS
- Glass coverslips No.1, 18mm
- Fixation buffer: 4% paraformaldehyde in 1xPBS
- Permeabilization buffer : 0.1% Triton X-100 in 1xPBS
- Blocking buffer: 5% Fetal Bovine Serum (FBS) in 1xPBS
- Fluorescence-labeled secondary antibody

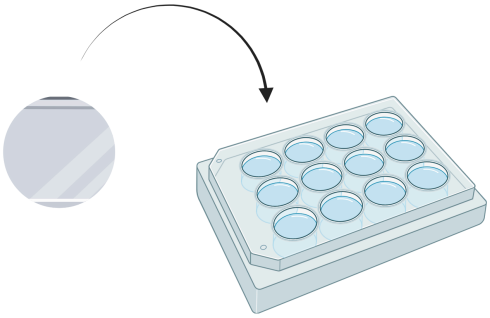
Fixation permeabilization

Steps

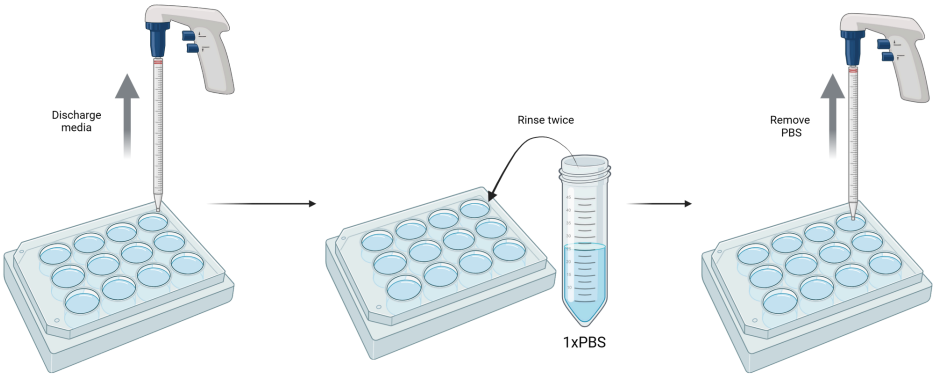
1 Coat the coverslips with 0.1 ng/ml poly-L-lysine solution at room temperature for 2 h, dry, and then wash with 1x PBS buffer.



2 Place the coated coverslip into each well of 12-well plate, and inoculate cells the day before immunocytochemistry experiment.

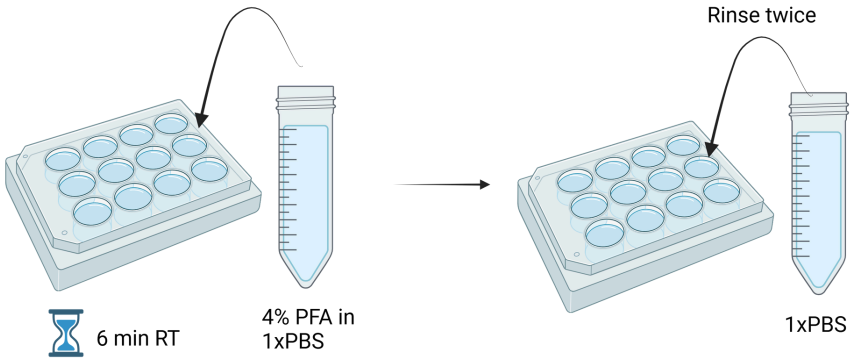


3 Discharge the medium and rinse cells attached to cover slips twice with 1x PBS, removing liquid by gentle aspiration in this and subsequent steps.



4

Fix cells with 4% paraformaldehyde in 1xPBS for 6 min at room temperature, and then rinse briefly twice with 1xPBS.



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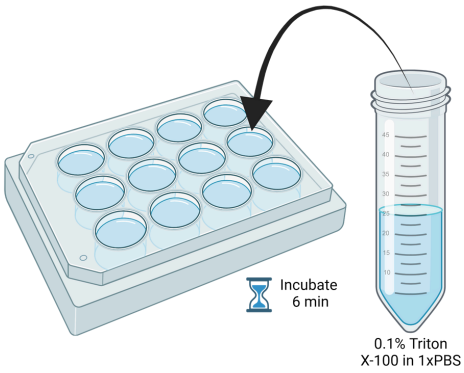
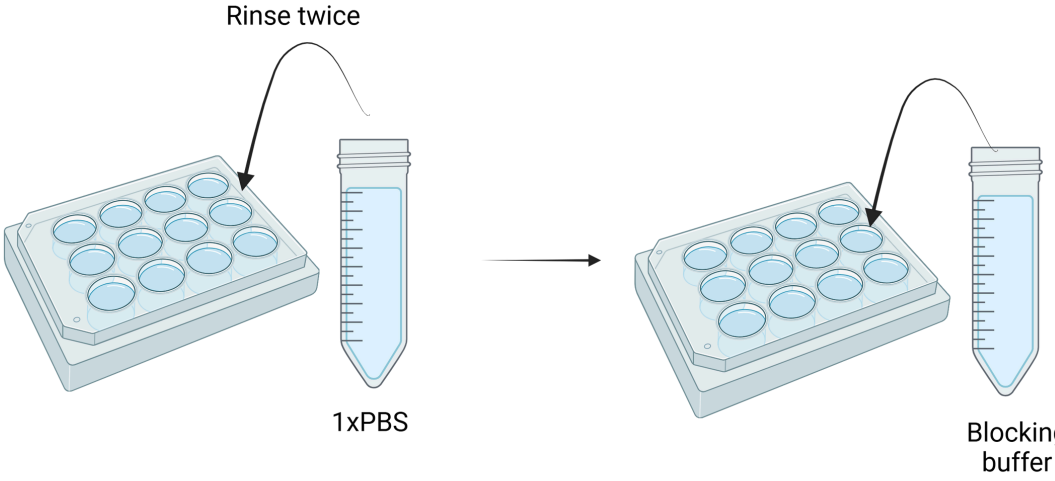
The fixed cells can be permeabilized with 0.1% Triton X-100 in 1xPBS for 6 min.



6

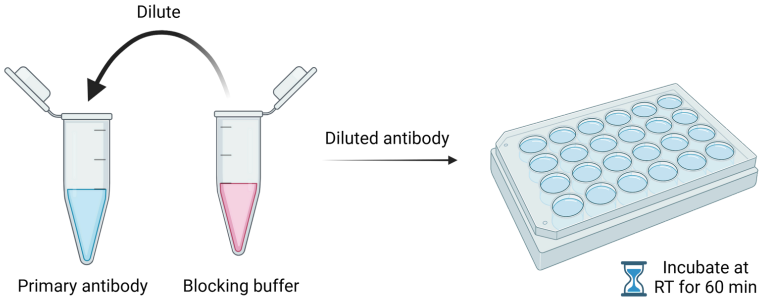
Wash cells briefly twice with 1x PBS, then block the coverslip with blocking buffer at room temperature.



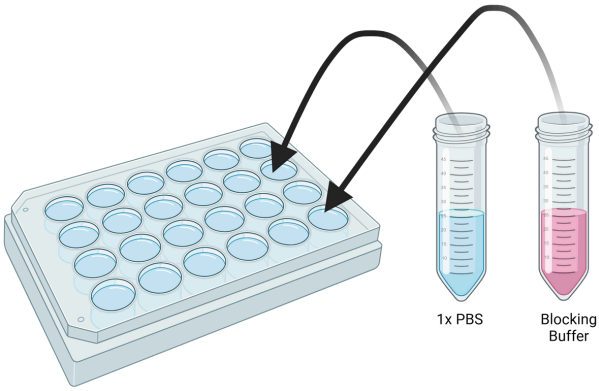
Staining

Steps

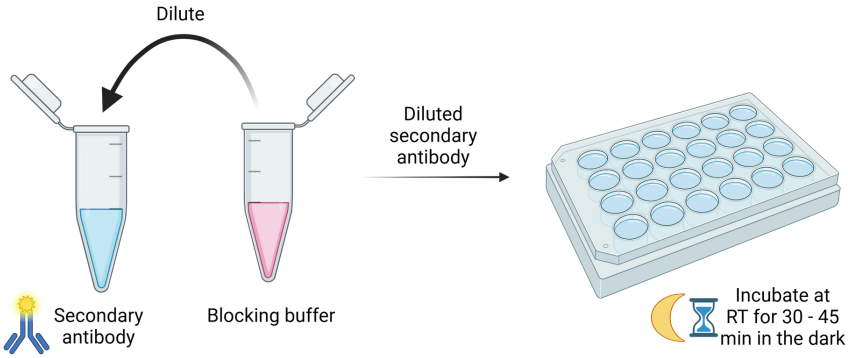
1 Dilute primary antibody with blocking buffer, and incubate the coverslip for 60 min at room temperature.



2 Wash cells three times with 1x PBS, then twice with blocking buffer.

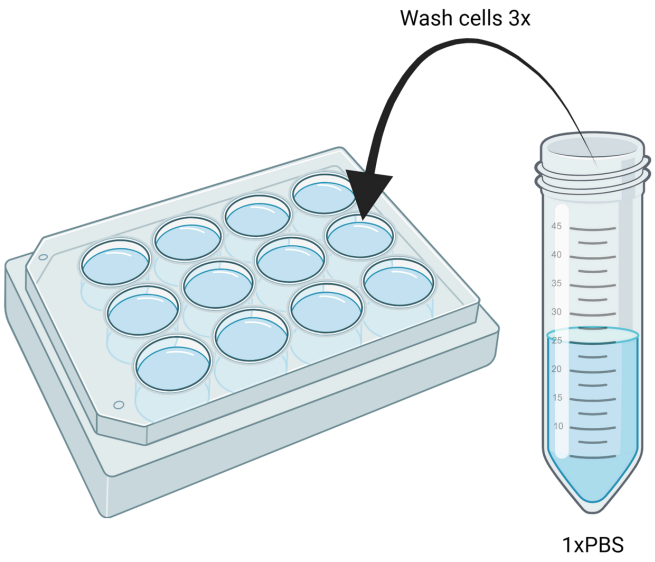


3 Incubate cells with a dilution of the fluorescence-labeled secondary antibody in blocking buffer for 30 - 45 minutes at room temperatures in the dark.



4

Wash cells three times with 1xPBS.



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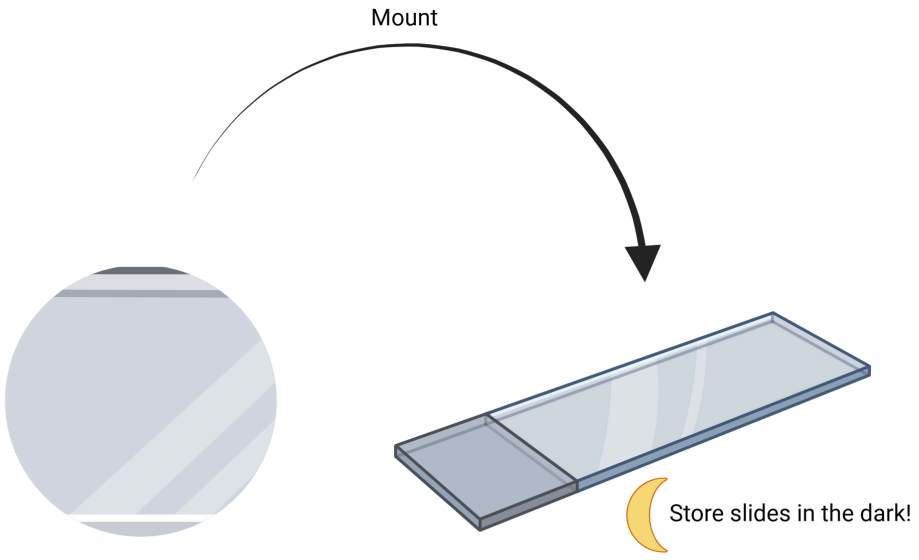
Mount the coverslip on a glass slide. Store the slides in the dark.



Immunofluorescent Staining Protocol Overview

Fixation permeabilization:

- Coat the coverslips with 0.1mg/ml poly-L-lysine solution at room temperature for 2h, dry, and then wash with 1xPBS buffer.
- Place the coated coverslip into each well of 12-well plate, and inoculate cells the day before immunocytochemistry experiment.
- Discharge the medium and rinse cells attached to cover slips twice with 1xPBS, removing liquid by gentle aspiration in this and subsequent steps.
- Fix cells with 4% paraformaldehyde in 1xPBS for 6 min at room temperature, and then rinse briefly twice with 1xPBS.
- The fixed cells can be permeabilize with 0.1% Triton X-100 in 1xPBS for 6 min.
- Wash cells briefly twice with 1xPBS, then block the coverslip with blocking buffer briefly at R/T.

Staining:

- Dilute primary antibody with blocking buffer, and incubate the coverslip for 60 min at room temperature.
- Wash cells 3 times with 1xPBS, then 2 times with blocking buffer.
- Incubate cells with a dilution of the fluorescence-labeled secondary antibody in blocking buffer for 30–45 minutes at room temperature in the dark.
- Wash cells three times with 1xPBS.
- Mount the coverslip on a glass slide. Store the slides in the dark.

Notes

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