# Immunofluorescence on Frozen Tissue Sections

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**[Abstract]** Immunofluorescence is commonly used to determine the cellular or tissue localization of a protein of interest. Immunofluorescence can also be used as a qualitative measure of protein expression.

## Materials and Reagents

1. Primary antibody
2. Alexa fluor secondary antibodies (Life Technologies, Invitrogen™)
3. Horse serum (Santa Cruz Biotechnology, catalog number: sc-2483)
4. Hoechst 33342 nuclear dye (Life Technologies, Invitrogen™, catalog number: H1399)
5. ProLong gold antifade mounting reagent (Life Technologies, Invitrogen™, catalog number: P36934)
6. PAP pen (Sigma-Aldrich, catalog number: Z377821)
7. Nail polish (Thermo Fisher Scientific, catalog number: 50-949-071)
8. 100% ice cold acetone
9. Phosphate buffered saline (PBS)
10. Formaldehyde
11. Methanol
12. Triton X-100

## Equipment

1. -20 °C freezer
2. Humidified chamber

## Procedure

1. Remove 7 µm cryosections from the freezer and immediately place them into ice-cold acetone for 10 min.
   *Note: The optimal fixation conditions depend upon the antigen and the primary antibody used. Formaldehyde, acetone and methanol are all common fixatives.*
2. Wash slides in PBS and aspirate around the tissue until the slide, not the tissue, is dry. Carefully, trace around the tissue with a PAP pen.

3. Block for 1 h at room temperature (RT) with 10% horse serum in PBS with 0.05% Triton X-100.
4. Aspirate blocking buffer and incubate sections in a humidified camber with primary antibody, diluted in 2% horse serum in PBS with 0.05% Triton X-100, for 1 h at RT. Use enough antibody solution to completely submerge the section, about 150 µl.
   *Note: Follow manufactures recommendation for antibody dilution.*
5. Aspirate primary antibody and wash in 150 µl of PBS/Triton X-100 buffer 5 times.
6. Incubate sections in a humidified chamber with corresponding secondary Alexa Fluor488/555 antibody for 1 h at RT. Dilute secondary in 2% horse serum in PBS with 0.05% Triton X-100, using a 1:300 ratio. Use enough antibody solution to completely submerge the section, about 150 µl.
7. Aspirate secondary antibody and wash in 150 µl of PBS/Triton X-100 buffer 2 times.
8. Wash sections in 150 µl of PBS buffer 3 times.
9. Incubate sections for 2 min in Hoechst 33342 nuclear dye [2 mg/ml in PBS].
10. Aspirate secondary antibody and wash in 150 µl of PBS buffer 3 times.
11. Allow sections to dry, add ~2 drops of ProLong gold antifade reagent to each section and place a cover slip over section.
    *Note: Be careful to avoid damaging the tissue by sliding the cover slip too much and do not introduce bubbles. Aspirate excess reagent.*
12. Dry sections at RT for several hours or overnight protected from light. Once the slides are completely dry, seal the edges of the cover slip with clear nail polish. Slides can be stored at -20 °C, protected from light for several weeks.