



# Immunofluorescence Protocol

**NOTE:** Prepare solutions with purified water.

## Section A: Buffers and Reagents

**Wash buffer:** PBS <http://www.fitzgerald-fii.com/pbs-10x-concentrate-85r-125.htm>

**Primary antibody:** See our selection of monoclonals (<http://www.fitzgerald-fii.com/monoclonal-antibodies.html>) or polyclonals (<http://www.fitzgerald-fii.com/polyclonal-antibodies.html>).

**Secondary antibody:** See our full list of conjugated secondary antibodies at <http://www.fitzgerald-fii.com/secondary-antibodies.html>, and choose by source animal, conjugated substance for your choice.

**Mounting media:** <http://www.fitzgerald-fii.com/mounting-media-99-med.html>

**Some other useful reagents:** <http://www.fitzgerald-fii.com/diluents.html>

**Reagents specific to IF-P application:**

### 1. Xylene

**2. Ethanol**, anhydrous denatured, histological grade, 100% and 95%.

### 3. Antigen Unmasking:

- a. **For Citrate:** 10 mM Sodium Citrate Buffer: To prepare 1 L add 2.94 g sodium citrate trisodium salt dihydrate ( $C_6H_5Na_3O_7 \cdot 2H_2O$ ) to 1 L dH<sub>2</sub>O. Adjust pH to 6.0.
- b. **For EDTA:** 1 mM EDTA: To prepare 1 L add 0.372 g EDTA ( $C_{10}H_{14}N_2O_8Na_2 \cdot 2H_2O$ ) to 1 L dH<sub>2</sub>O. Adjust pH to 8.0.

## Section B: Specimen Preparation

### I. Cultured Cell Lines (IF-IC)

**NOTE:** Cells should be grown, treated, fixed and stained directly in multi-well plates, chamber slides or on coverslips.

1. Aspirate liquid, then cover cells to a depth of 2–3 mm with 2-4 % paraformaldehyde in PBS
2. Allow cells to fix for 15 min at room temperature.
3. Aspirate fixative, rinse three times in PBS for 5 min each.
4. Proceed with Immunostaining (Section C).

### II. Paraffin Sections (IF-P)

**NOTE:** Do not allow slides to dry at any time during this process.

#### 1. Deparaffinization/Rehydration

- a. Incubate sections in three washes of xylene for 5 min each.
- b. Incubate sections in two washes of 100% ethanol for 10 min each.
- c. Incubate sections in two washes of 95% ethanol for 10 min each.
- d. Rinse sections twice in dH<sub>2</sub>O for 5 min each.

#### 2. Antigen Unmasking

**NOTE:** Consult product datasheet for specific recommendation for the unmasking solution.

- a. **For Citrate:** Bring slides to a boil in 10 mM sodium citrate buffer pH 6.0, then maintain at a sub-boiling temperature for 10 min. Cool slides on bench top for 30 min.
- b. **For EDTA:** Bring slides to a boil in 1 mM EDTA pH 8.0 followed by 15 min at a sub-boiling temperature. No cooling is necessary.

#### 3. Proceed with Immunostaining (Section C).

### III. Frozen/Cryostat Sections (IF-F)

1. For fixed frozen tissue proceed with Immunostaining (Section C).
2. For fresh, unfixed frozen tissue, please fix immediately, as follows:
  - a. Cover sections with 2-4% paraformaldehyde in PBS.
  - b. Allow sections to fix for 15 min at room temperature.
  - c. Rinse slides three times in PBS for 5 min each.
  - d. Proceed with Immunostaining (Section C).

### Section C: Immunostaining

#### General Procedure

**NOTE:** All subsequent incubations should be carried out at room temperature unless otherwise noted in a humid light-tight box or covered dish/plate to prevent drying and fluorochrome fading.

1. Block specimen in Blocking Buffer, 10% normal serum in PBS for 60 min.
2. While blocking, prepare primary antibody in PBS containing 2% normal serum and 0.1% TX100, by diluting as indicated on datasheet
3. Aspirate blocking solution, apply diluted primary antibody.
4. Incubate overnight at 4°C.
5. Rinse three times in PBS for 5 min each. **NOTE:** If using primary antibodies directly conjugated with fluorochromes, then skip to (Section C, Step 8).
6. Incubate specimen in fluorochrome-conjugated secondary antibody diluted in 2% normal serum in PBS containing 0.1% TX100, for 1–2 hr at room temperature in dark. ( May need to check several dilutions to optimize maximum signal and minimum background.
7. Rinse three times in PBS for 5 min each.
8. Coverslip slides with Anti-Fade Reagent
9. Ready to view under fluorescent microscope under appropriate wave length filters.
10. For long-term storage, store slides flat at 4°C protected from light.

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