Applications	Reactivity	Sensitivity	Isotype
IF-IC	H M Mk Dm Z B (C)	Endogenous	Rabbit IgG

Applications Key: IF-IC=Immunofluorescence (Immunocytochemistry)

Reactivity Key: H=Human M=Mouse Mk=Monkey C=Chicken Dm=D. melanogaster Z=Zebrafish B=Bovine

Species cross-reactivity is determined by western blot. Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Protocols

Immunofluorescence General Protocol

IMPORTANT: Please refer to the APPLICATIONS section on the front page of product datasheet to determine if this product is validated and approved for use on cultured cell lines (IF-IC), paraffin-embedded samples (IF-P), or frozen tissue sections (IF-F). Please see product datasheet for appropriate antibody dilution and unmasking solution.

A. Solutions and Reagents

NOTE: Prepare solutions with purified water.

1. **10X Phosphate Buffered Saline (PBS):** To prepare 1 L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phosphate, dibasic (Na_2HPO_4) and 2.4 g potassium phosphate, monobasic (KH_2PO_4) to 1 L dH_2O. Adjust pH to 8.0.
2. **Formaldehyde:** 16%, methanol free, Polysciences, Inc. (cat# 18814), use fresh, store opened vials at 4 ℃ in dark, dilute in PBS for use.
3. **Blocking Buffer:** (1X PBS / 5% normal goat serum (#5425) / 0.3% Triton™ X-100): To prepare 25 ml, add 2.5 ml 10X PBS, 1.25 ml normal serum from the same species as the secondary antibody (e.g., normal goat serum, normal donkey serum) and 21.25 ml dH_2O and mix well. While stirring, add 75 µl Triton™ X-100.
4. **Antibody Dilution Buffer:** (1X PBS / 1% BSA / 0.3% Triton™ X-100): To prepare 40 ml, add 4 ml 10X PBS and 120 µl Triton™ X-100 to 0.4 g BSA. Bring to final volume of 40 ml with dH_2O and mix well.
5. **Fluorochrome-conjugated secondary antibody NOTE:** When using any primary or fluorochrome-conjugated secondary antibody for the first time, titrate the antibody to determine which dilution allows for the strongest specific signal with the least background for your sample.
6. **Prolong® Gold Anti-Fade Reagent** (#9071), with DAPI (#8961).

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 ($C_6H_5Na_3O_7•2H_2O$) to 1 L dH_2O. Adjust pH to 6.0.

b. **For EDTA:** 1 mM EDTA: To prepare 1 L add 0.372 g EDTA ($C_{10}H_{14}N_2O_8Na_2•2H_2O$) to 1 L dH_2O. Adjust pH to 8.0.

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 4% formaldehyde in PBS.**NOTE:** Formaldehyde is toxic, use only in fume hood.
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_2O for 5 min each.

2. **Antigen Unmasking:**

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

2.

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 4% formaldehyde 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).

C. Immunostaining

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 for 60 min.
2. While blocking, prepare primary antibody by diluting as indicated on datasheet in Antibody Dilution Buffer.
3. Aspirate blocking solution, apply diluted primary antibody.
4. Incubate overnight at 4 ℃.

5. Rinse three times in PBS for 5 min each.

   NOTE: If using primary antibodies directly conjugated with Alexa Fluor® fluorochromes, then skip to (Section C, Step 8).

6. Incubate specimen in fluorochrome-conjugated secondary antibody diluted in Antibody Dilution Buffer for 1–2 hr at room temperature in dark.
7. Rinse in PBS (Section C, Step 5).
8. Coverslip slides with Prolong® Gold Anti-Fade Reagent (#9071), with DAPI (#8961).
9. For best results, allow mountant to cure overnight at room temperature. For long-term storage, store slides flat at 4 ℃ protected from light.

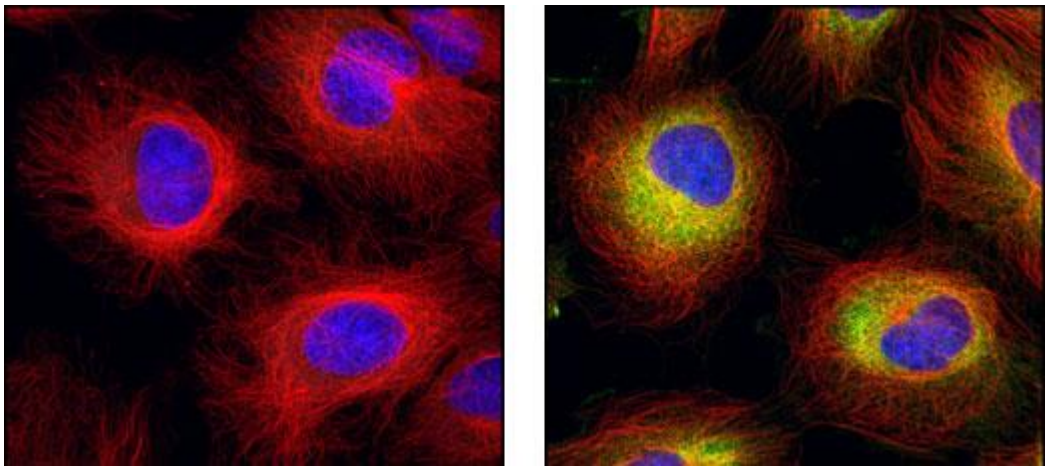
Specificity / Sensitivity

β-Tubulin (9F3) Rabbit mAb (Alexa Fluor® 555 Conjugate) detects endogenous levels of total β-tubulin protein.

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to the amino terminus of human β-tubulin. The antibody was conjugated to Alexa Fluor® 555 under optimal conditions with an F/P ratio of 2-6.

IF-IC



Confocal immunofluorescent analysis of HeLa cells, serum-starved (left) or 20% serum-treated (right), using β-Tubulin (9F3) Rabbit mAb (Alexa Fluor® 555 Conjugate) (red) and Phospho-S6 Ribosomal Protein (Ser235/236) (2F9) Rabbit mAb (Alexa Fluor® 488 Conjugate) #4854 (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

Description

Cell Signaling Technology antibody is conjugated to Alexa Fluor® 555 fluorescent dye and tested in-house for direct immunofluorescent analysis of human and monkey cells. The unconjugated antibody #2128 reacts with human, mouse, rat, monkey, bovine, zebrafish and fly β-tubulin protein. CST expects that β-Tubulin (9F3) Rabbit mAb (Alexa Fluor® 555 Conjugate) will also recognize β-tubulin in these species.