Applications	Reactivity	Sensitivity	Isotype
IF-IC F	All	Transfected Only	Mouse IgG2a

Applications Key: IF-IC=Immunofluorescence (Immunocytochemistry) F=Flow Cytometry

Reactivity Key: All=All species expected

Species cross-reactivity is determined by western blot.

Protocols

Flow Cytometry Protocol

A. Solutions and Reagents

NOTE: Prepare solutions with purified water.

1. **1X Phosphate Buffered Saline (PBS):** Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4in 800 ml dH_2O. Adjust the pH to 7.4 with HCl and the volume to 1 L. Store at room temperature.
2. **Formaldehyde** (methanol free).
3. **100% Methanol**
4. **Incubation Buffer:** Dissolve 0.5 g bovine serum albumin (BSA) in 100 ml 1X PBS. Store at 4 ℃.

B. Fixation

1. Collect cells by centrifugation and aspirate supernatant.
2. Resuspend cells briefly in 0.5–1 ml PBS. Add formaldehyde to a final concentration of 2–4% formaldehyde.
3. Fix for 10 min at 37 ℃.
4. Chill tubes on ice for 1 min.
5. For extracellular staining with antibodies that do not require permeabilization, proceed to Section D, Step 1 or store cells in PBS with 0.1% sodium azide at 4 ℃; for intracellular staining, proceed to permeabilization (Section C, Step 1).

C. Permeabilization

1. Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol. Alternatively, to remove fix prior to permeabilization, pellet cells by centrifugation and resuspend in 90% methanol.
2. Incubate 30 min on ice.
3. Proceed with immunostaining (Section D, Step 1) or store cells at –20 ℃ in 90% methanol.

D. Immunostaining

NOTE: Account for isotype matched controls for monoclonal antibodies or species matched IgG for polyclonal antibodies. Count cells using a hemocytometer or alternative method.

1. Aliquot 0.5–1x10^6 cells into each assay tube (by volume).
2. Add 2–3 ml Incubation Buffer to each tube and rinse by centrifugation. Repeat.
3. Resuspend cells in 100 µl Incubation Buffer per assay tube.
4. Block in Incubation Buffer for 10 min at room temperature.

5. Add the unconjugated, biotinylated, or fluorochrome-conjugated primary antibody at the appropriate dilution to the assay tubes (see individual antibody datasheet for the appropriate dilution).
6. Incubate for 1 hr at room temperature.
7. Rinse as before in Incubation Buffer by centrifugation.
8. If using a fluorochrome-conjugated primary antibody, resuspend cells in 0.5 ml PBS and analyze on flow cytometer; for unconjugated or biotinylated primary antibodies, proceed to immunostaining (Section D, Step 9).
9. Resuspend cells in fluorochrome-conjugated secondary antibody or fluorochrome-conjugated avidin, diluted in Incubation Buffer at the recommended dilution.
10. Incubate for 30 min at room temperature.
11. Rinse as before in Incubation Buffer by centrifugation.
12. Resuspend cells in 0.5 ml PBS and analyze on flow cytometer; alternatively, for DNA staining, proceed to optional DNA stain (Section E, Step 1).

E. Optional DNA Stain

1. Resuspend cells in 0.5 ml of DNA dye (e.g. Propidium Iodide (PI)/RNase Staining Solution #4087).
2. Incubate for at least 5 min at room temperature.
3. Analyze cells in DNA stain on flow cytometer.

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.

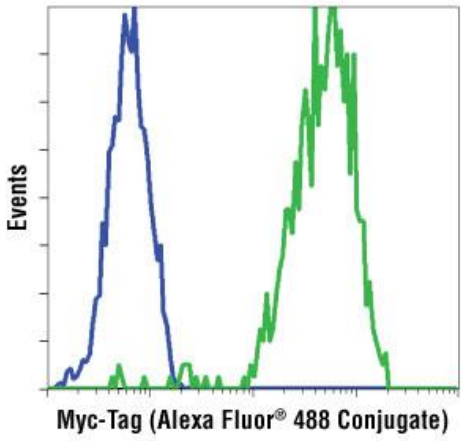
Specificity / Sensitivity

Myc-Tag (9B11) Mouse mAb (Alexa Fluor® 488 Conjugate) detects transfected proteins containing the Myc epitope tag. The antibody recognizes the Myc-tag fused to either the amino or carboxy terminus of targeted proteins in transfected mammalian cells.

Source / Purification

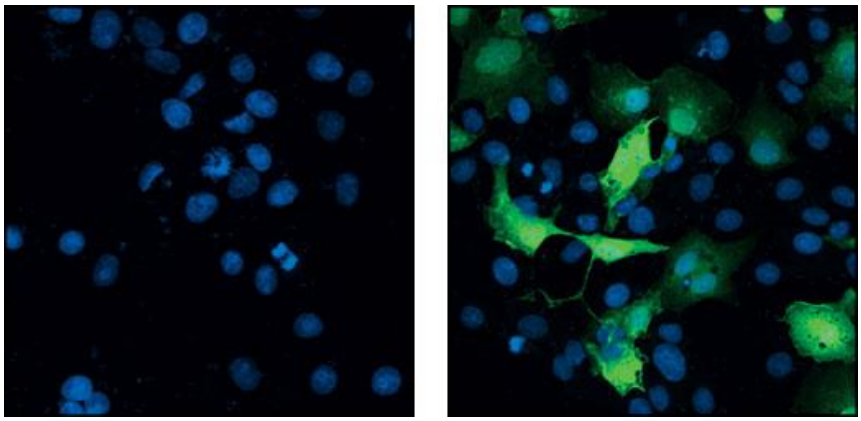
Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues 410-419 of human c-Myc (EQKLISEEDL). The antibody was conjugated to Alexa Fluor® 488 under optimal conditions with an F/P ratio of 2-6.

Flow Cytometry



Flow cytometric analysis of COS cells, untransfected (blue) or transfected with Myc-tagged Akt (green), using Myc-Tag (9B11) Mouse mAb (Alexa Fluor® 488 Conjugate).

IF-IC



Confocal immunofluorescent analysis of parental COS cells (left) and COS cells expressing a Myc-tagged protein (right), using Myc-Tag (9B11) Mouse mAb (Alexa Fluor® 488 Conjugate) (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).