

Phospho-Tyrosine Mouse mAb (P-Tyr-102) (Fluorescein Conjugate)

✓ 15 µg
(100 immunocytochemical stainings)

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This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

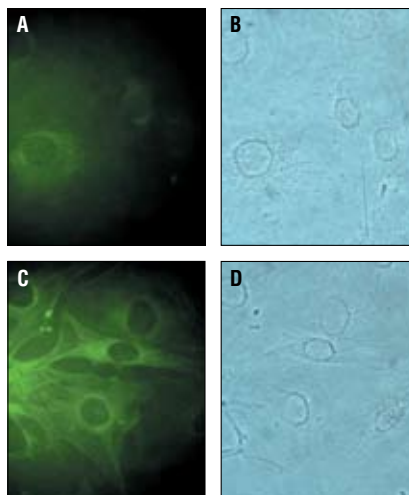
Applications	Species Cross-Reactivity	Source	Isotype
IF-IC, F	All	Mouse	IgG1

Background: Tyrosine phosphorylation plays a key role in cellular signaling (1). In cancer, unregulated tyrosine kinase activity can drive malignancy and tumor formation by generating inappropriate proliferation and survival signals (2). Antibodies specific for phospho-tyrosine (3,4) have been invaluable reagents in these studies. The phospho-tyrosine Mouse mAbs developed by CST (P-Tyr-100, #9411 and P-Tyr-102, #9416) provide exceptionally sensitive new tools of increased utility for studying tyrosine phosphorylation and monitoring tyrosine kinase activity in high throughput drug discovery.

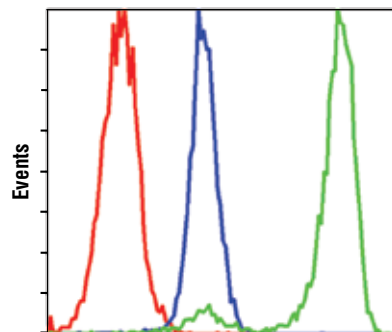
Specificity/Sensitivity: Phospho-Tyrosine Mouse mAb (P-Tyr-102) (Fluorescein Conjugate) is prepared by cross-linking fluorescein to the epsilon amine groups on lysines and amino-terminal amines. Fluorescein absorbs visible blue light at wavelength 490 nm and emits visible green light at 520 nm. This antibody is useful for immunocytochemical detection of tyrosine-phosphorylated proteins. It does not cross-react with proteins phosphorylated on threonine or serine. (U.S. Patent No's.: 6,441,140; 6,982,318; 7,259,022; 7,344,714; U.S.S.N. 11,484,485; and all foreign equivalents.)

Source/Purification: Monoclonal antibody is produced by immunizing mice with phospho-Tyr-containing peptides (KLH coupled). The antibody is purified by protein A chromatography.

License/Use Restrictions: Use of CST Motif Antibodies within certain methods (e.g., U.S. Patent No.'s 7,198,896 & 7,300,753) may require a license from CST. For information regarding academic licensing terms please have your technology transfer office contact CST Legal Department at CST_ip@cellsignal.com. For information regarding commercial licensing terms please contact CST Business Development at cbunker@cellsignal.com.



Immunocytochemical analysis of 10T1/2 cells, untreated (B) or sodium vanadate-treated (1 mM for 0.5 hour) (D), using Phospho-Tyrosine Mouse mAb (P-Tyr-102) (Fluorescein Conjugate). Images A and C are light micrographs of cells stained in B and D.



Flow cytometric analysis of Phospho-Tyrosine Mouse mAb (P-Tyr-102) (Fluorescein Conjugate) staining of untreated (blue) and pervanadate treated (green) NIH/3T3 cells compared with a nonspecific negative control antibody (red).

Storage: Supplied in PBS with 100 µg/ml BSA and 0.1% of sodium azide. Store at 4°C. Do not aliquot the antibody.

Recommended Antibody Dilutions:

Immunofluorescence (IF-IC) 1:1000
Flow Cytometry 1:50

Companion Products:

Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411
Phospho-Tyrosine Mouse mAb (P-Tyr-102) #9416
Phospho-Tyrosine Mouse mAb (P-Tyr-100) (Biotinylated) #9417
Immobilized Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9419
Phospho-Tyrosine Mouse mAb (P-Tyr-100) (Alexa Fluor® 488 Conjugate) #9414
Phospho-Tyrosine Mouse mAb (P-Tyr-100) (Alexa Fluor® 647 Conjugate) #9415

Background References:

- (1) Schlessinger, J. (2000) *Cell* 103, 211–225.
- (2) Blume-Jensen, P. and Hunter, T. (2001) *Nature* 411, 355–365.
- (3) Ward, S.G. et al. (1992) *J. Biol. Chem.* 267, 23862–23869.
- (4) Glenney, J.R. et al. (1988) *J. Immunol. Methods.* 109, 277–285.

Immunocytochemistry Protocol for Immunofluorescence

This procedure works well when cells are grown in 6-well tissue culture plates containing sterile coverslips in the appropriate media and concentration of fetal bovine serum (FBS). If desired, provide extra coverslips for cell staining controls.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. Fetal Bovine Serum (FBS)
2. **10X Phosphate Buffered Saline (PBS):** To prepare 1L 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 7.4.
3. **4% Paraformaldehyde:** Prepare day of use either from commercial 16% stock solution or by dissolving paraformaldehyde in 1X PBS by heating at 60°C and stirring until it dissolves. Cool before use.
4. **Tris Buffered Saline (TBS):** 50 mM Tris-HCl (pH 7.4), 150 mM NaCl.
5. **0.1% Sodium Borohydride:** Dissolve in PBS on day of use.
6. **0.2% Triton X-100:** Prepare stock of 20% Triton in PBS; rotate tube overnight to dissolve. Dilute to 0.2%.
7. 1% Bovine Serum Albumin (BSA)
8. **Blocking Buffer:** 10% horse or goat serum, 1% BSA, 0.02% NaN_3 , 1X PBS.
9. **ProLong™ Antifade Kit:** As directed by Molecular Probes (#P-7481), prepare just before use. Add approximately 1 ml of ProLong mounting medium to one vial of ProLong antifade reagent. Mix gently. Any unused mixture can be stored at -20°C (to slow reaction) for up to one month.

B Fixation

1. Prepare 4% paraformaldehyde and/or cool 100% methanol to -20°C in a tightly sealed container.
2. Treat cells as desired.
3. Wash cells on coverslips once with cool or room temperature TBS.
4. Aspirate off TBS completely and immediately fix as appropriate for your samples.

C Methanol (protein precipitation) Fixation

1. Immerse coverslips in -20°C 100% methanol for 10 minutes.
2. Rinse slips three times for 5 minutes each with room temperature TBS.
3. Quench cells in fresh 0.1% sodium borohydride in TBS for 5 minutes.
4. Wash slips three times for 5 minutes each with room temperature TBS. Aspirate off completely and return slips to TBS only. Continue protocol at "Blocking" step E1.

D Paraformaldehyde (cross-linking) Fixation

1. Immerse coverslips in 4% paraformaldehyde at room temperature for 10 minutes.
2. Wash coverslips once with TBS. Aspirate completely and then permeabilize cells on coverslips with 0.2% Triton X-100 for 5 minutes at room temperature or alternatively with -20°C methanol for 5 minutes.
3. Wash slips three times for 5 minutes each with TBS at room temperature.
4. Quench cells in fresh 0.1% sodium borohydride in TBS for 5 minutes. Aspirate off completely and return slips to TBS only. Continue protocol at blocking step.

E Blocking

1. Block all slips with blocking buffer at room temperature for 45–60 minutes. Wash once for 5 minutes with TBS.

F Staining

1. Dilute the primary antibody as appropriate in 1% BSA in TBS. Centrifuging the antibody for 20 minutes at 12,000 x g in a refrigerated microcentrifuge prior to use will remove any aggregated material, thereby reducing background. Apply the diluted antibody to the cells on coverslips and, most critically, incubate overnight at 4°C.

NOTE: When using any primary or fluorescence-labeled secondary antibody for the first time, titrate out the antibody to determine which dilution allows for the strongest specific signal with the least background for your sample.

NOTE: You may wish to leave one slip for a secondary antibody-only control.

2. Wash all slips three times for 5 minutes each with TBS.
3. Incubate all slips with a dilution of the fluorescence-labeled secondary antibody in 1% BSA in TBS for 30–45 minutes at room temperature in the dark.
4. Wash all slips three times for 5 minutes each with TBS in low lighting conditions.
5. Mount coverslips on slides using the ProLong™ Antifade Kit. Store slides at room temperature in the dark.

Flow Cytometry Protocol for Intracellular Staining Using Conjugated Secondary Antibodies

A Solutions and Reagents

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_4 in 800 mL distilled water (dH_2O). Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at room temperature.
2. Formaldehyde (methanol free)
3. **Incubation Buffer:** Dissolve 0.5 g bovine serum albumin (BSA) in 100mL 1X PBS. Store at 4°C

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 minutes at 37°C.
4. Chill tubes on ice for 1 minute.

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 minutes on ice.
3. Proceed with staining or store cells at -20°C in 90% methanol.

D Staining Using Unlabeled Primary and Conjugated Secondary Antibodies

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

1. Aliquot 0.5-1x10⁶ 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 minutes at room temperature.
5. Add the primary antibody at the appropriate dilution to the assay tubes (see individual antibody data sheet for the appropriate dilution).
6. Incubate for 30-60 minutes at room temperature.
7. Rinse as before in Incubation Buffer by centrifugation.
8. Resuspend cells in fluorochrome-conjugated secondary antibody*, diluted in Incubation Buffer according to the manufacturer's recommendations.
9. Incubate for 30 minutes at room temperature.
10. Rinse as before in Incubation Buffer by centrifugation.
11. Resuspend cells in 0.5 ml PBS and analyze on flow cytometer.

*Recommended Secondary Antibodies from Invitrogen.

A-11070 Alexa Fluor® 488 F(ab')₂ fragment of goat anti-rabbit IgG (H+L) (1:1000 dilution)

A-11017 Alexa Fluor® 488 F(ab')₂ fragment of goat anti-mouse IgG (H+L) (1:1000 dilution)