



PN A24073 – 50 tests – Liquid – 0.5 mL – Clone P-Tyr-100

**For Research Use Only. Not for use in diagnostic procedures.**

### SPECIFICITY

Since the original observations that protein tyrosine kinases (PTKs) play critical roles in oncogenic transformation (1, 2), it has become clear that both protein tyrosine kinases and phospho-tyrosine phosphatases control a wide range of critical biological processes (3, 4). Antibodies specific for phospho-tyrosine (5, 6) have been invaluable reagents in these studies. The phospho-tyrosine monoclonal antibody (P-Tyr-100) provides exceptionally sensitive new tool of increased utility for studying tyrosine phosphorylation and monitoring tyrosine kinase activity in high throughput drug discovery.

The anti-Phospho-Tyrosine Mouse mAb (P-Tyr-100) is a high affinity antibody. ELISA assays against a wide variety of phosphopeptides indicate that (a) P-Tyr-100 binds phospho-Tyr in a manner largely independent of the surrounding amino acid sequence, and (b) P-Tyr-100 binds to a larger number of phospho-Tyr-containing peptides and does not cross-react with peptides containing phospho-Ser or phospho-Thr. (Patented, U.S. No. 6,441,140 and Patents Pending).

### REAGENT

Phospho-Tyrosine (P-Tyr-100) - Alexa Fluor 647

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<b>Clone</b>	P-Tyr-100
<b>Isotype</b>	IgG1
<b>Immunogen</b>	phospho-tyrosine-containing peptides (KLH-coupled)
<b>Species</b>	Mouse
<b>Purification</b>	Ion exchange chromatography or chromatography on protein A

### Conjugation

Alexa Fluor 647 under optimum conditions with a F/P ratio of 2 – 6.  
Excitation wavelength: 650 nm  
Max emission wavelength: 668 nm  
Main emission color: Far Red

**Buffer** 2 mg/mL bovine serum albumin in Phosphate-Buffered saline containing 0.1% sodium azide.

### APPLICATION

This reagent is designed for Flow Cytometry analysis of permeabilized cells. It is usable in Image cytometry analysis.

### STATEMENT OF WARNINGS

1. This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes
4. Do not use antibody beyond the expiration date on the label.
5. Do not expose reagents to strong light during storage or incubation.
6. Avoid microbial contamination of reagents or incorrect results might occur.
7. Use good laboratory practices when handling this reagent.

### STORAGE CONDITIONS AND STABILITY

This reagent is stable up to the expiration date when stored at 2 – 8°C. Do not freeze. Minimize exposure to light.

### REAGENT PREPARATION

No reconstitution is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 18 – 25°C prior to use.

### PROCEDURE

#### Flow Cytometry for Intracellular Staining

##### 1. Solutions and Reagents

- 1X Phosphate-Buffered Saline (PBS): Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g

Na<sub>2</sub>HPO<sub>4</sub> and 0.24 g KH<sub>2</sub>PO<sub>4</sub> in 800 mL distilled water. Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at 18 – 25°C.

- Formaldehyde (methanol free).
- Incubation Buffer: Dissolve 0.5 g bovine serum albumin (BSA) in 100 mL 1X PBS. Store at 2 – 8°C.

##### 2. Fixation

- Collect cells by centrifugation and aspirate supernatant.
- Resuspend cells briefly in 0.5 – 1 mL of PBS, then add formaldehyde so that the final concentration is 1% – 2% formaldehyde.
- Fix for 10 minutes at 37°C.
- Chill tubes on ice for 1 minute.

##### 3. Permeabilization

- Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells while gently vortexing so that final concentration is 90% methanol. Alternatively, to remove fix prior to permeabilization, centrifuge and resuspend cell pellet in 90% methanol.
- Incubate 30 minutes on ice or at 2 – 8°C.
- Proceed with staining or store cells at – 20°C in 90% methanol.

##### 4. Staining

Note: for each sample analyzed, in addition to the assay tube, one control tube is required in which the cells are mixed in the presence of the appropriate isotypic control.

- Count cells using hemacytometer or alternative method.
- Aliquot 5x10<sup>5</sup> cells into each assay tube.
- Add 2 – 3 mL of Incubation Buffer to each tube and rinse by centrifugation.
- Resuspend cells in 90 µL of Incubation Buffer per assay tube.
- Let cells block in Incubation Buffer for 10 minutes at room temperature.
- Add 10 µL of conjugated antibody to the assay tubes.

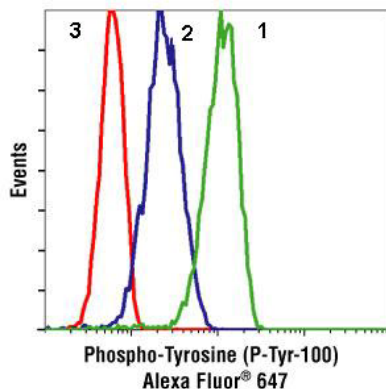


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- Incubate for 30 – 60 minutes, in the dark at room temperature.
- Rinse as before in Incubation Buffer by centrifugation.
- Resuspend cells in 0.5 mL of PBS and analyze on flow cytometer.

#### EXAMPLE DATA

Flow cytometry analysis of K562 cells, untreated (1) or Gleevec™ -treated (2), using Alexa Fluor 647-conjugated Phospho-Tyrosine Mouse (P-Tyr-100) (Clone P-Tyr-100) compared with a nonspecific negative control antibody (3).  
Data provided by Cell Signaling Technology, Inc.



#### REFERENCES

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3. Fischer, E.H., "Cell signaling by protein tyrosine phosphorylation", 1999, *Adv. Enzyme Regul.*, 39, 359–369.
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5. Ward, S.G., Reif, K., Ley, S., Fry, M.J., Waterfield, M.D., Cantrell, D.A., "Regulation of phosphoinositide kinases in T cells. Evidence that phosphatidylinositol 3-kinase is not a substrate for T cell antigen receptor-regulated tyrosine kinases", 1992, *J. Biol. Chem.*, 267, 23862–23869.
6. Glenney, J.R., Zokas, L., Kamps, M.P., "Monoclonal antibodies to phosphotyrosine", 1988, *J. Immunol. Methods*, 109, 277–285.

#### PRODUCT AVAILABILITY

Phospho-Tyrosine (P-Tyr-100) - Alexa Fluor 647  
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#### OTHER FORMS AVAILABLE

Phospho-Tyrosine (P-Tyr-100) - Alexa Fluor 488

PN A24072 – 50 tests – 0.5 mL

For additional information in the USA, call 800-526-7694.

Outside the USA, contact your local Beckman Coulter representative.

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