



CELL LAB Mouse Anti-Phosphotyrosine

<u>Cat. No.</u>	<u>Form</u>	<u>Quantity</u>
732483	Purified (UNLB) Antibody	0.5 mg
732484	Alkaline (AP) Phosphatase Conjugate	1.0 mL
732485	Horseradish (HRP) Peroxidase Conjugate	1.0 mL
732486	Biotin (BIOT) Conjugate	0.5 mg

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

DESCRIPTION

Clone: PY20
Isotype: Mouse IgG2b κ
Specificity: Phosphorylated tyrosine and phenylphosphate

Protein tyrosine residues are phosphorylated as a result of intracellular protein kinase activation (for example, via growth factors) during normal growth and development, and in oncogenesis. The most abundant population of target proteins for tyrosine phosphorylation are cell surface glycoproteins. Antibody to phosphotyrosine enables the detection, isolation and characterization of proteins containing phosphotyrosine.¹ PY20 also prevents internalization of activated receptors, for example, EGFr, when microinjected into cells.³ The affinity of PY20 for phosphotyrosine is approximately 10^{-6} to 10^{-7} M.² PY20 binding to phosphorylated tyrosines can be inhibited by free phosphotyrosine and phenylphosphate, but not by phosphoserine, phosphothreonine or free phosphate.¹

APPLICATIONS

- Detection of phosphorylated tyrosine-containing proteins on Western blots¹
- Enzyme-Linked Immunosorbent Assay (ELISA) for quantitation of phosphotyrosine¹
- Immunofluorescence analysis⁴

CHARACTERIZATION

To ensure lot-to-lot consistency, each batch of product is tested to conform with characteristics of a standard reference reagent using ELISA.

WORKING DILUTIONS

Western Blotting:	Purified antibody	2 μ g/mL
ELISA:	Purified antibody	100 ng/mL
	AP conjugate	1:500-1:1,000
	HRP conjugate	1:1,000-1:4,000

Other Applications: Since applications vary, determine the optimum working dilution of the product that is appropriate for your specific needs.

HANDLING AND STORAGE

- The purified (UNLB) antibody is supplied as 0.5 mg of purified immunoglobulin in 1.0 mL of 100 mM borate buffered saline, pH 8.0. No preservatives or amine-containing buffer salts added.
- The alkaline phosphatase (AP) conjugate is supplied as 1.0 mL of stock solution in 50% glycerol/50% 50 mM Tris, pH 8.0, containing 0.1% NaN₃ as preservative.
- The horseradish peroxidase (HRP) conjugate is supplied as 1.0 mL of stock solution in 50% glycerol/50% PBS, pH 7.4. No preservative added.
- The biotin (BIOT) conjugate is supplied as 0.5 mg in 1.0 mL of PBS/NaN₃.
- Reagent is stable until the expiration date on the vial when stored at 2-8°C.

STATEMENT OF WARNINGS

1. 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.
2. Never pipet by mouth and avoid contact of samples with skin and mucous membranes.
3. Do not use reagent beyond the expiration date on the vial label.
4. Avoid microbial contamination of reagent or erroneous results may occur.
5. Use Good Laboratory Practice (GLP) when handling this reagent.
6. Harmful if swallowed.
7. After contact with skin, wash immediately with plenty of water.
8. Contains sodium azide. Sodium azide under acidic 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, immediately wash excessively with water.

TRADEMARKS

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For additional information or if damaged product is received, contact your local Beckman Coulter Representative.

REFERENCES

1. Glenney JR, Jr., Zokas L and Kamps MP. 1988. Monoclonal antibodies to phosphotyrosine. *J Immunol Methods*, 109:277-285.
2. Ruff-Jamison S, Campos-Gonzalez R and Glenney JR, Jr. 1991. Heavy and light chain variable region sequences and antibody properties of anti-phosphotyrosine antibodies reveal both common and distinct features. *J Biol Chem*, 266:6607-6613.
3. Glenney JR, Jr., Chen WS, Lazar CS, Walton GM, Zokas LM, Rosenfeld MG and Gill GN. 1988. Ligand-induced endocytosis of the EGF receptor is blocked by mutational inactivation and by microinjection of anti-phosphotyrosine antibodies. *Cell*, 52:675-684.
4. Takagi S, Daibata M, Last TJ, Humphreys RE, Parker DC and Sairenji T. 1991. Intracellular localization of tyrosine kinase substrates beneath cross linked surface immunoglobulins in B cells. *J Exp Med*, 174:381-388.



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