



Qty: 100 µg/200 µl

Mouse anti-
Phosphothreonine

Catalog No. 13-9200

Lot No.

Mouse anti-Phosphothreonine

FORM

This antibody is supplied as a 200 µl aliquot of antibody at a concentration of 0.5 mg/ml in PBS (pH 7.4) containing 0.1% NaN₃ as a preservative. The antibody is purified from mouse ascites by phosphothreonine-specific, affinity-chromatography.

CLONE: PT-5H5

ISOTYPE: IgG_{2a}-kappa

IMMUNOGEN: Phosphothreonine containing proteins.

SPECIFICITY

PT-5H5 reacts specifically with proteins containing phosphorylated threonine residues (phosphothreonine). Recognition of phosphothreonine containing proteins by this antibody appears to be independent of neighboring amino acids and species of origin of the phosphorylated protein. No significant cross-reactivity with phosphoserine, phosphotyrosine either as free amino acids or in proteins has been observed. Specific inhibition of anti-phosphothreonine reactivity is achieved by pre-incubation of the antibody with 20 mM phosphothreonine; 20 mM phosphoserine or 20 mM phosphotyrosine are ineffective.

REACTIVITY

Recognition of phosphothreonine containing proteins by this antibody appears to be independent of the species of origin of the phosphorylated protein. This antibody has been confirmed to react with phosphothreonine containing proteins from lysates derived from EGF-stimulated A431 cells, HeLa cells, MCF-7 cells and Clone 9 (rat liver) cells.

USAGE

The concentrations given below are good starting points; however, optimal dilution of the antibody should be determined by the investigator for each application. When using this antibody it is important to recognize that the accessibility of the phosphothreonine residue(s) within the native protein, and possibly the extent of protein phosphorylation are likely to influence the effectiveness of this antibody in your particular assay system.

Western Blotting⁽⁸⁻¹¹⁾: 1-5 µg/ml (see note)

ELISA: 0.1-1.0 µg/ml

STORAGE

Store at 2-8°C for up to one month. Store at -20°C for long term storage. Avoid repeated freezing and thawing.

BACKGROUND

Reversible protein phosphorylation plays a central role in numerous biochemical pathways and functions to alter protein activity and/or conformation.^(3,7) Methods for detecting protein phosphorylation have predominately relied upon the use of radioactive ³²P for either the *in vitro* or *in vivo* phosphorylation reaction.^(2,6) To identify the specific amino acid residues that become phosphorylated on a specific protein, phosphoamino acid analysis is then required.^(2,3,6,7) The use of radioactivity and the multi-step analysis process which follows makes the entire process both hazardous and tedious.

Over the past decade, antibodies specific for phosphotyrosine were developed, many of which are can detect a single phosphorylated tyrosine residue.^(1,4,5) Although antibodies to phosphotyrosine have been relatively straight forward to produce, the extensive structural similarity between phosphoserine and phosphothreonine has contributed to the difficulty in raising highly specific antibodies to these phosphoamino acids.⁽⁶⁾ Invitrogen has employed painstaking schemes of antigen design and antibody purification to derive our monoclonal anti-phosphothreonine antibodies which we believe offer the highest performance of any competing product available today.

(cont'd)

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NOTE

Milk-derived blocking solutions reportedly contain phosphoproteins that may inhibit phosphoamino acid antibody binding and therefore should be avoided. Invitrogen's Membrane Blocking Solution (Cat. No. 00-0105) is optimized for use with anti-phosphoamino acid antibodies and provides enhanced blocking of non-specific signal. A 3% BSA (bovine serum albumin) solution may also be used.

REFERENCES

1. Frackelton, AR, et al; *Mol. Cell. Biol.* 3:1343 (1983).
2. Edelman, AM, et al; *Annu. Rev. Biochem.* 56:567 (1987).
3. Hunter, T. *Cell* 50:823 (1987).
4. Glenn, JR, et al; *J. Immunological Meth.* 109:277 (1988).
5. Sengupta, A, et al; *Proc. Natl. Acad. Sci. USA* 85:8062 (1988).
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8. Rintamäki, E. et al; *J. Biol. Chem.* 272(48): 30476-30482 (1997).
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10. Reilein A.R., et al; *J. Biol.* 142(3):803-813 (1998).
11. Fleming, I.N., et al; *J. Biol. Chem.* 274(18):12753-12758 (1999).

RELATED PRODUCTS

Product	PAD*/clone	Cat. No.	
Phosphothreonine Ab Inhibitor	----	79-0002	
Rb x Phosphothreonine	Z-PT1	71-8200	
Rb x PS/PT/PY (pan)	polyclonal	61-8300	
Phospho-Amino Acid Sampler Pack (pSer, pThr, PY-Plus™ Cocktail)	3 antibodies	90-0200	
Phosphotyrosine Sampler Pack	6 antibodies	90-0100	
Ms x Phosphoserine	PT-5H5	61-8100	
Phosphoserine Ab Inhibitor	----	79-0001	
Rb x Phosphotyrosine	Z-PY1	61-5800	
Rb x Phosphotyrosine-HRP	Z-PY1	61-5820	
Rb x Phosphotyrosine-Sepharose®	Z-PY1	61-5841	
Ms x Phosphotyrosine	PY-7E1	13-5900	
Ms x Phosphotyrosine-HRP	PY-7E1	13-5920	
Msx Phosphotyrosine	PY-1B2	13-6300	
Ms x Phosphotyrosine	PY20	03-7700	
Ms x Phosphotyrosine (1 mg size)	PY20	03-7799	Excellent Value!
Ms x Phosphotyrosine-HRP	PY20	03-7720	
Ms x Phosphotyrosine-AP	PY20	03-7722	
Ms x Phosphotyrosine-Biotin	PY20	03-7740	
Ms x Phosphotyrosine- Sepharose®	PY20	03-7742	
Ms x Phosphotyrosine	Z027	03-5800	
Phosphotyrosine Ab inhibitor	---	79-0003	
PY-Plus™ Cocktail	3 mabs	13-6600	
PY-Plus™ Cocktail-HRP	3 mabs	13-6620	
Protein A	Sepharose® 4B	10-1041	
rec-Protein G	Sepharose® 4B	10-1241	

Conjugate	ZyMAX™ Goat x Rabbit IgG (H+L)	ZyMAX™ Goat x Mouse IgG (H+L)
Purified	81-6100	81-6500
FITC	81-6111	81-6511
TRITC	81-6114	81-6514
Cy™3	81-6115	81-6515
Cy™5	81-6116	81-6516
HRP	81-6120	81-6520
AP	81-6122	81-6522
Biotin	81-6140	81-6540

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