



Qty: 50 µg/200 µl

Rabbit anti-Phosphothreonine

Catalog No. 71-8200

Lot No.

Rabbit anti-Phosphothreonine

FORM

This polyclonal antibody is supplied as a 200 µl aliquot at a concentration of 0.25 mg/ml in PBS (pH 7.4) containing 0.1% sodium azide (NaN₃). The antibody was purified from rabbit antiserum by phosphothreonine-specific affinity chromatography.

POLYCLONAL ANTIBODY DESIGNATION (PAD): Z-PT1

IMMUNOGEN

Phosphothreonine containing proteins.

SPECIFICITY

This antibody reacts specifically with proteins containing phosphorylated threonine residues (phosphothreonine). Recognition of phosphothreonine containing proteins by this antibody is independent of neighboring amino acids and species of origin of the phosphorylated protein. This antibody shows no significant cross-reactivity to either phosphoserine or phosphotyrosine.

REACTIVITY

This antibody detects phosphothreonine bands on lysates derived from NIH 3T3 cells (+/- TPA), K562 cells, and EGF-stimulated A431 cells. Specific inhibition of anti-phosphothreonine reactivity is achieved by pre-incubation of the antibody with 20 mM phosphothreonine, whereas 20 mM phosphoserine or 20 mM phosphotyrosine are ineffective.

USAGE

The dilutions 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: 1-2 µg/ml (see note)
Immunoprecipitation: 5-10 µg
ELISA: 0.1-1.0 µg/ml

Note

Milk-derived blocking solutions reportedly contain phosphoproteins that may inhibit phosphoamino acid antibody binding and therefore should be avoided. Zymed'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.

STORAGE

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

(cont'd)

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BACKGROUND ⁽¹⁻⁷⁾

Reversible protein phosphorylation plays a central role in numerous biochemical pathways and functions to alter protein activity and/or conformation.^(5,6) Methods for detecting protein phosphorylation have predominately relied upon the use of radioactive ³²P for either the *in vitro* or *in vivo* phosphorylation reaction.^(1,4) To identify the specific amino acid residues that become phosphorylated on a specific protein, phosphoamino acid analysis is then required.^(1,4,5,6) The use of radioactivity and the multi-step analysis process which follows makes the entire process both hazardous and tedious.

Over the past ten years, antibodies which specifically recognize phosphotyrosine have been successfully developed.^(2,3) Further, many of these phosphotyrosine specific antibodies are able to detect even a single phosphorylated tyrosine residue.^(2,3,7) 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.⁽⁴⁾ Zymed has employed painstaking schemes of antigen design and antibody purification to derive our high quality polyclonal anti-phosphothreonine antibody, and we believe that this antibody is the best of its kind currently available on the market.

REFERENCES

1. Edelman, AM, et al; *Annu. Rev. Biochem.* 56:567 (1987).
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CITATIONS**Polyclonal Rabbit anti-pThr (Cat. No. 71-8200)****Application**

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Kamiguti, A., et al., <i>J. Biol. Chem.</i> 272: 32599-32605 (1997).	WB
Rintamäki, E., et al., <i>J. Biol. Chem.</i> 272: 30476-30482 (1997).	WB
Wilson, J.R., and T. Biden, <i>J. Biol. Chem.</i> 273: 22729-22737 (1998).	WB
Yuan QX, et al., <i>Exp Mol Pathol</i> 65:87-103 (1998).	WB, IHC
Mahoney, C.W., et al., <i>Anal. Biochem.</i> 268:371-6 (1999).	ELISA

RELATED PRODUCTS

Product	PAD*/clone	Cat. No.	
Rb x Phosphoserine	Z-PS1	61-8100	
Phosphoserine Ab Inhibitor	----	79-0001	
Rb x PS/PT/PY (pan)	polyclonal	61-8300	
Phosphotyrosine Sampler Pack	6 antibodies	90-0100	
Ms x Phosphothreonine	PT-5H5	13-9200	
Phosphothreonine Ab Inhibitor	----	79-0002	
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	
Ms x 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 Clones	13-6600	
PY-Plus™ Cocktail-HRP	3 Clones	13-6620	

PAD: Polyclonal Antibody Designation

Protein A	Sepharose® 4B	10-1041
rec-Protein G	Sepharose® 4B	10-1241

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