



Qty: 50 µg/200 µl

Rabbit anti-Phosphoserine

Catalog No. 61-8100

Lot No.

## Rabbit anti-Phosphoserine

### FORM

This antibody is supplied as a 200 µl aliquot at a concentration of 0.25 mg/ml in PBS (pH 7.4) containing 0.1% NaN<sub>3</sub>. This polyclonal antibody was purified from rabbit antiserum by phosphoserine-specific, affinity chromatography.

**PAD (Polyclonal Antibody Designation):** Poly-Z-PS1

### IMMUNOGEN

Phosphoserine containing proteins.

### SPECIFICITY

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

### REACTIVITY

Reactivity of this antibody has been confirmed by Western blotting and specific cross reactivity is confirmed by ELISA against phosphotyrosine, and phosphothreonine. Specific inhibition of anti-pSer reactivity is achieved by pre-incubation of the antibody with 20 mM phosphoserine (cat. no. 79-0001); incubation with 20 mM phosphothreonine or 20 mM phosphotyrosine shows no inhibition. Positive control: NIH 3T3 cells (+/- TPA), K562 cells, EGF-stimulated A431 cell lysates.

### BACKGROUND<sup>1-7</sup>

Reversible protein phosphorylation plays a central role in numerous biochemical pathways and functions to alter protein conformation and activity.<sup>(6,7)</sup> Detection of protein phosphorylation is frequently accomplished by incorporation of exogenous <sup>32</sup>P<sup>(1,5)</sup> followed by amino acid analysis.<sup>(1,5,6,7)</sup> In an effort to simplify this process, specific antibodies to Phosphotyrosine (PY) were initially developed<sup>(3,4)</sup>, some with the ability to detect a single phosphorylated tyrosine residue.<sup>(3,4,8)</sup> While the production of anti-phosphotyrosine antibodies has been relatively straight forward, the structural similarity of phosphoserine (PS) and phosphothreonine (PT) has made production of specific antibodies to these amino acids more problematic.<sup>(5)</sup> Invitrogen has developed proprietary antigen design and antibody purification techniques to produce our anti-phosphoserine antibody. We believe Invitrogen's phosphoserine antibody to be the best available.

### USAGE

The dilutions given below are good starting points; however, an 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 phosphoserine residue(s) within the native protein and possibly the extent of protein phosphorylation can influence their ability to bind proteins.

|  |                    |
|--|--------------------|
| <b>Western Blotting*:</b>                      | 1-2 µg/ml          |
| <b>ELISA*:</b>                                 | 0.1-1.0 µg/ml      |
| <b>Immunoprecipitation (IP)<sup>(2)</sup>:</b> | 3-5 µg/IP reaction |

\* See REFERENCES below for published research citing the use of this product.

### 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.*

(continued)

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**STORAGE**

This antibody can be stored at 2-8°C for at least one month. For long term storage, -20°C is recommended; however, repeated freezing and thawing should be avoided.

**REFERENCES**Background References

1. Edelman, AM., et al., Annu. Rev. Biochem. 56:567 (1987).
2. Fleming IN, et al. J. Biol. Chem. 272(52):33105-33110 (1997).
3. Frackelton, AR., et al., Mol. Cell. Biol. 3:1343 (1983).
4. Glenn, JR., et al., J. Immunological Meth. 109:277 (1988).
5. Heffetz, D., et al., Methods in Enzymology 210:44 (1991).
6. Hunter, T. Methods in Enzymology 200, 3 (1991).
7. Hunter, T Cell 50, 823 (1987).
8. Sengupta, A., et al., Proc. Natl. Acad. Sci. USA 85:8062 (1988).

Invitrogen Rabbit anti-Phosphoserine Citations (Immunoblotting)

Fleming, I., et al., J. Biol. Chem. 272:33105-33110 (1997).  
 Kamiguti, A., et al., J. Biol. Chem. 272:32599-32605 (1997).  
 Yagi, K., et al., J. Biol. Chem. 274:703-709 (1997).  
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 Jin, D-Y, et al., Cell 93:81-91 (1998).  
 Kawabata, M., et al., EMBO J. 17: 4056-4065 (1998).  
 Nishimura, R., et al., J. Biol. Chem. 273:1872-1879 (1998).  
 Reilein, A.R., et al., J. Cell Biol., 142:803-813 (1998).  
 Tuvia, S., et al., J. Cell Biol., 141:1551-1561 (1998).

Invitrogen Rabbit anti-Phosphoserine Citations (ELISA)

Mahoney, C.W., et al., Anal. Biochem. 268:371-6 (1999).

**RELATED PRODUCTS**

| <b>Product</b>                   | <b>PAD*clone</b> | <b>Cat. No.</b> |
|----------------------------------|------------------|-----------------|
| Phosphoserine Ab Inhibitor       | ----             | 79-0001         |
| Phospho-Amino Acid Sampler Pack  | 3 antibodies     | 90-0200         |
| Rb x PS/PT/PY (pan)              | polyclonal       | 61-8300         |
| (pSer, pThr, PY-Plus™ Cocktail)  |                  |                 |
| Rb x Phosphothreonine            | Z-PT1            | 71-8200         |
| Ms x Phosphothreonine            | PT-5H5           | 13-9200         |
| Phosphothreonine Ab Inhibitor    |                  | 79-0002         |
| Phosphotyrosine Sampler Pack     | 4 antibodies     | 90-0100         |
| 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             | PY20             | 03-7700         |
| Ms x Phosphotyrosine (1 mg size) | PY20             | 03-7799         |
| 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         |
| Phosphotyrosine Ab inhibitor     | ---              | 79-0003         |
| PY-Plus™ Cocktail                | 3 mabs           | 13-6600         |
| PY-Plus™ Cocktail-HRP            | 3 mabs           | 13-6620         |
| <b>Product</b>                   | <b>Conjugate</b> | <b>Cat. No.</b> |
| Goat anti-Rabbit IgG (H+L)       | Purified         | 81-6100         |
| (ZyMAX™ Grade)                   | FITC             | 81-6111         |
|                                  | TRITC            | 81-6114         |
|                                  | Cy™3             | 81-6115         |
|                                  | Cy™5             | 81-6116         |
|                                  | HRP              | 81-6120         |
|                                  | AP               | 81-6122         |
|                                  | Biotin           | 81-6140         |
| Protein A                        | Sepharose® 4B    | 10-1041         |
| rec-Protein G                    | Sepharose® 4B    | 10-1241         |

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