ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Anti-Phosphothreonine Antibodies and control

Cat. PTHR12-M	Monoclonal Anti-Phosphothreonine, ascites, unconjugated	**SIZE**: 100 ul
Cat. PTHR12-BTN	Monoclonal Anti-Phosphothreonine IgG-Biotinylated	**SIZE**: 100 ul
Cat. PTHR15-N	Phosphothreonine-BSA control (blocking protein)	**SIZE**: 100 ug

Protein phosphorylation and dephosphorylation reactions is a key postranslational event in the modification of protein functions. Protein phosphorylation occurs at tyrosine, serine, or threonine (p-tyr, p-ser, or p-thr) residues. Many different mitogenic systems, such as the EGF, PDGF, and insulin receptor systems contain tyr/ser/thr kinase domains which autophosphorylate specific tyr, ser or thr residues upon binding of their ligands. T cell antigen receptor complex or the receptors for some hemopoietic growth factors may stimulate associated kinases, and cells transformed by viral oncogenes contain elevated levels of phosphorylated proteins. An understanding of transformation by oncogenes and mitogenic processes of growth factors requires a delineation of all potential partners involved in phosphorylation cascade. The availability of antibodies specific for p-tyr, p-thr or p-thr residues has greatly advanced the studies on role of phosphorylated proteins.

Source of Antigen and Antibodies

Antigen	o-Phospho-L-threonine-conjugated to KLH
Ab Host/type	Mouse, monoclonal, ascites designated as # PTHR12-M (isotype IgG2b), supplied in PBS+0.05% azide
2-Ab	**Goat Anti-mouse IgG-HRP conjugate** Cat # 40320 (AP, biotin, FITC conjugates also available)
-ve control IgG	Cat # 20008-1, Mouse (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as –ve control

Cat# PTHR12-BTN, Biotin-conjugate

Purified anti-phosphothreonine IgG (#PTHR12-M) was coupled to Biotin using Biotinamidocaproate N-Hydroxysuccinimide Ester (BAC) at F/P ratio ~10-20:1. The antibody is supplied in PBS, pH 7.4, 0.2% BSA and 0.05% azide in either **lyophilized** or **liquid** form (0.05-0.1 mg/ml). Reconstitute powder in PBS in 0.1 ml to prepare stock solution. Store at -20oC in suitable aliquots. Stability is ~6-12 months. Do not freeze and thaw.

Suggested conjugate dilutions are 1:5,000-1:30,000 ELISA, 1:2K-1:10K for western.

Form & Storage of Antibodies/Peptide Control

Mouse Ascites (unpurified)
100ul solution powder
Supplied in Buffer: 0.05% azide
Reconstitute powder in 100 ul PBS

Storage
Short-term: unopened, undiluted liquid vials at -20OC and powder at 4oC or -20oC..
Long-term: at –20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at –20oC or below.
Shipping: 4oC for solutions and room temp for powder

Phosphothreonine-BSA Control (blocking antigen) #PSER15-N

O-phosphothreonine was conjugated to bovine serum albumin (BSA) to serve as positive control (**Cat# PTHR15-N**) for anti-phosphothreonine (#PTHR12-M) in ELISA or Western or as used blocking protein. **Cat # Cat# PTHR15-N**) is supplied in PBS, pH 7.4 at 1 mg/ml in solution or in powder (dissolve in 100 ul PBS to make 1 mg/ml). SDS-PAGE sample buffer (reduced). Use 2-10 ul of control per 1-2 ul of antibody for complete inhibition. For some applications, it may be necessary to optimize the concentration of both antibody and antigen (control) for optimal inhibition effect.

Use 1-5 ug/ml of #PTHR15-N for coating ELISA plates or use 1-2 ug for Western.

Suggested applications for antibodies

ELISA: use at 1:1K-1:5K
Western: use at 1:500-1:2K
IHC: 1-200-1:1K

Specificity & Cross-reactivity

Monoclonal Anti-Phosphothreonine (#PTHR12-M) reacts with phosphorylated threonine both as free amino acid or when conjugated to carriers such as BSA or KLH using ELISA and dot blot. No significant reactivity is observed with nonphosphorylated threonine, phosphorylated tyrosine or serine, AMP, or ATP. The antibody may be used for the immunolocalization of most phosphothreonine containing proteins using western or IHC. However, some proteins phosphorylated at threonine may not be recognized by this antibody due to steric hindrance of the recognition site.

General References: (1) Hunter, T. (1985) Annu. Rev. Biochem., 54, 897; Heffetz, D. (1991) Enzymol., 201, 44; Alexander, D.et al (1989) Immunol. Today, 10, 200; Levine, L., et al., (1989) J. Immunol. Methods, 124, 239

*This product is for *in vitro* research use only.

Related material available from ADI:

Anti-phospho-serine, phospho-tyrsoine, and phospho-threonine

Western Blot recycling kit (Use the same blot to probe with multiple antibodies CSP11, CLO11, etc.) **recycle blot at room temp in 5-10 min**; No mercaptoethanol or heating required).

PTHR12-M-BTN-C 71015A

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