ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Anti-Phosphotyrosine Antibodies and control

Cat. PTYR12-M	Monoclonal Anti-phosphotyrosine, unconjugated	**SIZE**: 100 ug
Cat. PTYR12-BTN	Monoclonal Anti-phosphotyrosine IgG-Biotinylated	**SIZE**: 100 ul
Cat. PTYR12-HRP	Monoclonal Anti-phosphotyrosine IgG-HRP Conjugate	**SIZE**: 100 ul
Cat. PTYR15-N	Phosphotyrosine-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-tyrosine-conjugated to KLH
Ab Host/type	Mouse, monoclonal, designated as # PTYR12-M (isotype IgG2b, clone PY20), Protein A/G purified IgG supplied in PBS+50% glycerol and 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# PTYR12-BTN, Biotin-conjugate

Purified anti-phosphotyrosine IgG (#PTYR12-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.

Cat# PTYR12-HRP, HRP-conjugate

Purified antibody was coupled to HRP (RZ>3.0) using periodate method. The molar enzyme to protein (E/P) ratio = 4.0. The antibody is supplied in stabilizing buffer, 0.1% prolcin-300 as preservative in either **lyophilized** (0.1 ml) or **liquid** form (0.5-0.1 mg/ml). Reconstitute powder in PBS in 0.1 ml. Store at 4oC in suitable aliquots. Stability is ~6-12 months. Do not freeze and thaw.

Suggested conjugate dilutions are 1:2,000-1:10,000 ELISA, 1:1K-1:5K for western, and 1:200-1:1000 (IHC).

Phosphotyrosine-BSA Control (blocking antigen) #PTYR15-N

O-phosphotyrosine was conjugated to bovine serum albumin (BSA) to serve as positive control (**Cat# PTYR15-N**) for anti-phosphotyrsoine (#PTYR12-M) in ELISA or Western or used as blocking protein. **Cat # Cat# PTYR15-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 #PTYR15-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-Phosphotyrosine (#PTYR12-M) reacts with phosphorylated tyrosine 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 tyrsoine, phosphorylated serine or threonine, AMP, or ATP. The antibody may be used for the immunolocalization of most phosphotyrosine containing proteins using western or IHC. However, some proteins phos phorylated at serine 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).

PTYR12-M-BTN-C 71015A

Alpha Diagnostic Intl Inc., 6203 Woodlake Center Dr, San Antonio, TX 78244, USA; **Email**: service@4adi.com
☎ (800) 786-5777; (210) 561-9515; **Fax**: (210) 561-9544; **Web Site**: www.4adi.com