

Anti-Phosphotyrosine

(rabbit polyclonal IgG)

Catalog # 06-427

Lot # 19078

Immunogens: In order to produce broad spectrum polyclonal phosphotyrosine antibodies, rabbits were immunized with three phosphorylated immunogens: (1) phosphotyrosine covalently linked to KLH; (2) the c-Src carboxyl terminal regulatory phosphopeptide (T-S-T-E-P-Q-pY-Q-P-G-E-N-L; Catalog # 12-218) covalently linked to KLH, and; (3) a phosphopeptide associated with high tyrosine kinase activity in human lymphocytes (R-R-L-I-E-D-A-E-pY-A-A-R-G; Catalog # 12-217) covalently linked to KLH. Both of the phosphopeptide haptens serve as strong substrates for tyrosine phosphatases and are part of the two colorimetric tyrosine phosphatase kits provided by Upstate Biotechnology, Inc. (Catalog # 17-125, 17-126).

Species Cross-reactivity: Human, mouse and rat. Other species cross-reactivity is unknown.

Specificity and Purification: The immunoreactivity of the antibody is totally inhibited by the use of 100mM phenyl phosphate, a phosphotyrosine analog. The phosphotyrosine antibody is purified by immunoaffinity chromatography using either a dual phospho-peptide gel or a BSA-phosphotyrosine gel. All of the phosphotyrosine immunoreactivity present in the antisera is immunoabsorbed whether the antibody is purified by either gel indicating that the antibody is not sequence-specific but specific for phosphotyrosine residues.

Formulation: 200ng of immunoaffinity purified rabbit IgG in 909ml of 0.2M Tris-glycine, pH 7.2, 0.15M NaCl, 1mg/ml of BSA containing 0.05% sodium azide. Concentration: 0.22mg/ml. Frozen solution.

Storage and Stability: Stable for 1 year at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing. For maximum recovery of the product, centrifuge the original vial after thawing and prior to removing the cap.

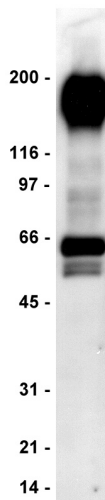
**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

Immunoblot Analysis: 0.5-1µg/ml of this lot detected proteins containing phosphotyrosine residues in RIPA lysates from EGF-stimulated human A431 carcinoma cells.

Included Positive Antigen Control: Catalog # 12-302, EGF-stimulated A431 cell lysate. Use 20µg per lane for minigels.

Immunoprecipitation: 4µg of this lot immunoprecipitated proteins containing phosphotyrosine residues from a human A431 RIPA lysate.



Immunoblot Analysis

EGF-stimulated A431 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Phosphotyrosine (0.5µg/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system.

References:

1. The cSrc carboxyl terminal regulatory phosphopeptide (T-S-T-E-P-Q-pY-Q-P-G-E-N-L) which binds to the internal SH2 domain of c-Src.
Song, Z., *et al.*, Cell **72**:767, 1993.
Luttrel, D.K., *et al.*, Proc. Natl. Acad. Sci. USA **91**: 83, 1994.
2. The phosphopeptide associated with high tyrosine kinase activity in human lymphocytes (R-R-L-I-E-D-A-E-pY-A-A-R-G).
Trevillyan, J.M., *et al.*, Biochim. Biophys. Acta **845**: 1, 1985.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1 μ g/ml aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS prepared PBS containing 0.05% Tween-20 and 3% nonfat dry milk (PBS-T-MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **0.5-1mg/ml of anti-Phosphotyrosine**, diluted in freshly prepared PBS-T-MLK, overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a **goat anti-rabbit** IgG linked to horseradish peroxidase, 1:3000 dilution, was used) in PBS-T-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

Immunoprecipitation Protocol

1. Prepare 500 μ g to 1mg of cell lysate at a concentration of 1 μ g/ μ l and add to a microcentrifuge tube.
2. Add **4mg of anti-Phosphotyrosine** to the tube.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 μ l of washed Protein A agarose bead slurry (50 μ l packed beads).
5. Gently rock the reaction mixture at 4°C for 2 hours.
6. Collect the agarose beads by pulsing 5 seconds in the microcentrifuge at 14,000 x g, and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in 50 μ l 2X Laemmli sample buffer.
8. The agarose beads can either be frozen for later use or suspended in Laemmli sample buffer and boiled for 5 minutes. The beads are collected by a microcentrifuge pulse and SDS-PAGE and subsequent immunoblot analysis can be performed on a sample of the supernatant.