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Product Information

Anti-Phosphotyrosine

Developed in Rabbit, Affinity Isolated Antibody

Product Number **T 1325**

Product Description

Anti-Phosphotyrosine is developed in rabbit using phospho-L-tyrosine conjugated to KLH as immunogen. Anti-Phosphotyrosine is affinity-purified using phospho-L-tyrosine immobilized on agarose.

Anti-Phosphotyrosine recognizes tyrosine phosphorylated proteins. The antibody recognizes phospho-L-tyrosine, but does not recognize phospho-L-serine, phospho-L-threonine and L-tyrosine as determined by inhibition in immunoblotting. The product is useful for immunoblotting and immunocytochemistry

Protein phosphorylation is the most abundant among post-translational modifications of cellular proteins, regulating intracellular signal transduction pathways in every living cell. Tyrosine, serine and threonine are the major amino acids that are phosphorylated in proteins. Tyrosine phosphorylation is a dynamic post-translational event in normal tissues regulating cell growth and development, and in oncogenesis.¹⁻⁴ Tyrosine phosphorylation accounts for only about 0.03% of phosphorylated amino acids in non-stimulated cells. However, this phosphorylation can increase several fold by various activation signals and the process is mediated by protein tyrosine kinases (PTKs).^{1,2} PTKs can be divided into two major groups, receptor tyrosine kinases (RTKs) and non-receptor tyrosine kinases (NRTKs).¹⁻² The RTK family includes many growth factor receptors (GFRs) such as the insulin-R, EGFR, PDGFR, FGFR and NGFR. These receptors possess an extra-cellular domain that is responsible for ligand binding, a trans-membrane domain and an intracellular domain that contains the catalytic activity and a regulatory sequence. The NRTK family consists of modular domains responsible for sub-cellular targeting and regulation of catalytic activity. This family includes Src, Abl, Fak, Jak and many others. The PTKs are responsible for many biological processes like cell cycle, proliferation, oncogenesis and development.

They are tightly regulated by other kinases and by autophosphorylation activity.^{1-2, 5,6} Antibodies specific for phosphotyrosine are essential tools for the characterization of tyrosine phosphorylation in many signal transduction pathways. Polyclonal antibodies can offer advantages over monoclonals, especially in immunoprecipitation protocols, and can theoretically react with a broader range of phosphotyrosine-containing proteins.

Reagents

The antibody is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, and 1% BSA, containing 15 mM sodium azide as a preservative.

Antibody Concentration: Approx. 0.5 mg/ml.

Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability

For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

A working dilution of 0.3-0.6 µg/ml is determined by immunoblotting using total cell extract of A431 stimulated by human EGF.

A working dilution of 3-6 µg/ml is determined by immunofluorescence using A431 stimulated by human EGF.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

References

1. Schlessinger, J., Cell, **103**, 211-225 (2000).
2. Blume-Jensen, P., and Hunter, T., Nature, **411**, 355-365 (2001).
3. Yarden, Y., and Sliwkovsky, M.X., Nat. Rev. Mol. Cell Biol., **2**, 127-137 (2001).
4. Schlaepfer,, D.D. and Hunter, T., Trends Cell Biol., **8**, 151-157 (1998).
5. Hubbard, S.R., et al., J. Biol. Chem., **273**, 11987-11990 (1998).
6. Zou, X., and Calame, K., J. Biol. Chem., **274**, 18141-18144 (1999).

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