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Product datasheet for R1178

Phosphothreonine Mouse Monoclonal Antibody [Clone ID: 18F6]

Product data:

Product Type:	Primary Antibodies
Clone Name:	18F6
Applications:	ELISA, WB
Recommended Dilution:	Phosphorylation of threonine residues is associated with many growth factors and oncogene protein kinases, and is important for cell signaling in activation, proliferation and differentiation. This monoclonal antibody reacts specifically with phosphothreonine and shows minimal reactivity by ELISA and competitive ELISA with phosphoserine or phosphotyrosine. The antibody reacts with free phospho amino acid, phosphothreonine conjugated to carriers such as thyroglobulin or BSA, and detects the presence of phosphothreonine in proteins of both unstimulated and stimulated cell lysates. Although not tested, this antibody is likely functional in RIA, Flow cytometry, Immunohistochemistry and Immunoprecipitation. Recommended Dilutions: This product was assayed by against phosphothreonine conjugated BSA by ELISA using HRP Goat-anti-Mouse IgG for detection. A working dilution of 1/2,000-1/10,000 is suggested for this assay. For Immunoblotting dilute the antibody 1:1000 immediately before use.
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	This monoclonal antibody was produced after repeated immunizations of balb/c mice with phosphotyrosine conjugated KLH.
Specificity:	This product is clarified ascites produced in balb/c mice using clone 18F6. Reactivity is specific for phosphothreonine and minimal cross reactivity is observed against phosphoserine or phosphotyrosine.
Formulation:	State: Ascites State: Liquid (sterile filtered) ascites with 0.01% sodium azide as preservative.

Conjugation: Unconjugated

Storage: Store the antibody (undiluted) at 2-8°C for one month or (in aliquots) at -20°C for longer.
Dilute only prior to immediate use.
Avoid repeated freezing and thawing.

Stability: Shelf life: One year from despatch.

Background: Protein phosphorylation and dephosphorylation are basic mechanisms for the modification of protein function in eukaryotic cells. Phosphorylation is a rare post-translational event in normal tissue, however, the abundance of phosphorylated cellular proteins increases several fold following various activation processes which are mediated through phosphotyrosine, phosphoserine or phosphothreonine (p-tyr/p-ser/p-thr). Many signal transduction pathways, such as the EGF, PDGF and insulin receptor systems, contain tyr/ser/thr kinase which phosphorylate specific tyr/ser/thr residues upon binding of ligands to their receptors. T cell antigen receptor complex or the receptors for some hemopoietic growth factors may stimulate these phosphorylation associated kinases, and cells transformed by viral oncogenes contain elevated levels of phosphorylated tyr/ser/thr. An understanding of transformation by oncogenes and mitogenic processes of growth factors depends on the identification of their substrate and a subsequent determination of how phosphorylation affects their properties. Studies on the role of phosphorylated proteins have been hampered by their low abundance and the problem of distinguishing the various types of phosphorylated proteins. The most common procedure is to label intact cells or small tissue fragments with 32P and subsequently to isolate 32P labeled proteins by conventional biochemical methods. In order to identify the specific amino acids that undergo phosphorylation, additional long and tedious procedures for phosphoamino acid analysis are required. Immunoblotting of cellular proteins with antibodies directed against phosphoamino acids is advantageous as it does not involve 32P labeling, and can therefore be employed to monitor alterations in phosphorylation of specific proteins as they occur in intact organs or the whole animal. Indeed, mono and polyclonal antibodies directed against phosphorylated residues have been generated and found useful as analytical and preparative tools because they enable the rapid identification, quantification and immunoaffinity isolation of phosphorylated cellular proteins.

Product images:

Figure 2. Mab anti-phosphothreonine antibody (clone 18F6) is shown to detect threonine phosphorylation of proteins in a lysate after EGF stimulation of A431 cells. Separation is achieved under reducing conditions using a pre-cast 4-20% iGel from Gradipore

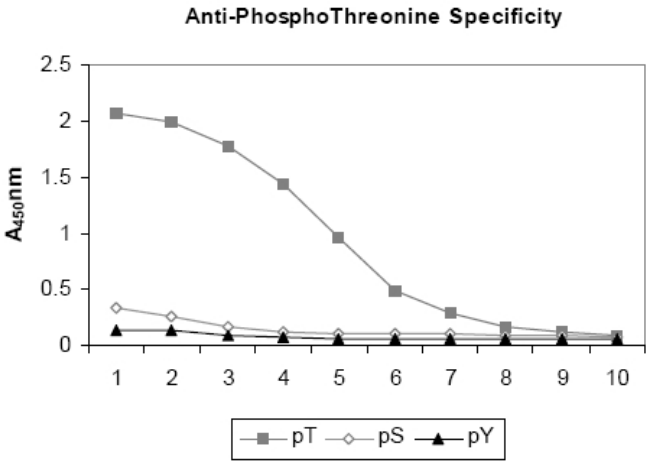

Figure 1. ELISA results of Mab antiphosphothreonine antibody tested against BSA conjugates of pT, pY and pS. Each well was coated with 0.1g of conjugate. The starting dilution of antibody was 1:1000 and each point on the Y-axis represents a 2-fold dilution