Bovine/Canine/Chicken/Primate Phospho-Raf-1 (S301) Antibody

Antigen Affinity-purified Polyclonal Rabbit IgG
Catalog Number: PPS060

DESCRIPTION

Species Reactivity	Bovine/Canine/Chicken/Primate
Specificity	Human, mouse, rat, and Xenopus ~73 kDa Raf-1 in Western blots.
Source	Polyclonal Rabbit IgG
Purification	Antigen Affinity-purified
Immunogen	Phosphopeptide corresponding to amino acid residues surrounding the phospho-S301 of Raf-1
Formulation	100 mL in 10 mM HEPES (pH 7.5), 150 mM NaCl, 100 mg/mL BSA and 50% glycerol. See Certificate of Analysis for details.

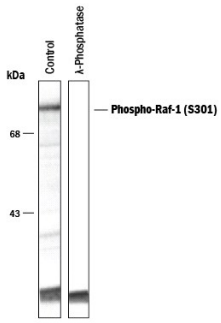
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	1:1000 dilution	See Below

DATA

Western Blot



Detects Phospho-Raf-1 (S301) by Western Blot. Western blot of UV treated human Jurkat cell lysate showing specific immunolabeling of the approximately 73 kDa Raf-1 protein (Control). The phosphospecificity of this labeling is shown in the second lane (lambda-phosphatase: l PPase). The blot is identical to the control except that it was incubated in l PPase (1200 units for 30 minutes) before being exposed to the anti-phospho-Raf-1 (S301). The immunolabeling of Raf-1 is completely eliminated by treatment with l PPase.

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	For long-term storage, ≤ -20° C is recommended. Product is stable at ≤ -20° C for at least 1 year.

BACKGROUND

Raf-1 (also C-Raf) is a ubiquitously expressed, 73 kDa, 648 amino acid (aa) Raf subfamily, Ser/Thr protein kinase family enzyme. Raf-1 is an entry point into the MAP kinase/Erk-1/2 signaling pathway. The molecule contains three distinct regions; an N-terminal RBD (Ras-binding domain) (amino acids 56-131), followed by two rich segments [a cysteine-finger region (amino acids 138-184) (also called CR1/C1) and a second cysteine-rich region (CR2) (amino acids 253-264)] and a C-terminal Ser/Thr kinase catalytic domain (amino acids 354-611). In general, Raf-1 phosphorylates and activates MEK-1/2. MEK-1/2, in turn, phosphorylates and activates ERK-1/2. The activation of Raf-1 begins upstream at the plasma membrane with the activation of Ras. During Ras activation, Raf-1 is inactive, in part because a 14-3-3 protein constitutively binds to phosphorylated Ser 259 and Ser 621 residues on Raf-1. Once activated, Ras binds Raf-1 on the Raf RBD, and displaces 14-3-3, allowing for additional Ras binding to the CR1. These two ligations now activate Raf-1. In the inactive state, Raf-1 is constitutively phosphorylated on Ser 43, Ser 259, and Ser 621. Upon activation, Ser 338, Tyr 431, Tyr 491, and Ser 494 also become phosphorylated, with phosphorylation at Ser 338 inducing activation. Down-regulation of Raf-1 occurs on other sites. Phosphorylation on proline-directed serine sites at position 301 and 642 are posited to be part of a negative feedback system regulated by ERK.

References:

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