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ProductInformation

ANTI-PHOSPHO-BAD (PHOSPHOSERINE 112)
Developed in Sheep,
Affinity Isolated Antibody

Product Number **B 5679**

Product Description

Anti-phospho-Bad (Phosphoserine 112) is developed in sheep using a synthetic peptide [RSRHS(pS)YPAGT] as immunogen. This sequence corresponds to amino acids 107-117 of mouse Bad. Whole sheep antiserum is purified using protein G and immunoaffinity chromatography to provide affinity isloated antibody.

Anti-phospho-Bad (Phosphoserine 112) recognizes mouse pS112 Bad (~23 kDa) and cross-reacts with human by immunoblotting. Due to low levels of endogenous phospho-Bad, immunoprecipitation of the total Bad is recommended prior to immunoblotting.

The Bcl-2 family of proteins contains anti- and pro-apoptotic molecules and is a critical, intracellular decision point in a common cell death pathway.[1] The ratio of anti- (Bcl-2, Bcl-x_L, Mcl-1, and A1) to pro- (Bax, Bak, Bcl-x_S, and Bad) apoptotic molecules dictates whether a cell will respond to a proximal apoptotic stimulus.[1,2] Bad, initially identified by its interaction with Bcl-2 and Bcl-x_L, is a distant Bcl-2 family member. It bears only the most universally conserved amino acids within BH1 and BH2 domains, and lacks the typical hydrophobic C-terminal signal-anchor. The presence of Bad counters the anti-apoptotic effect of Bcl-x_L or Bcl-2.[3] Bad interconnects signal transduction pathways from extracellular survival factors with the Bcl-2 intracellular checkpoint for cell death. Bad is phosphorylated on two serine residues embedded in canonical 14-3-3 binding sites in response to a survival factor, IL-3.[1] Phosphorylated Bad does not bind Bcl-x_L and is sequestered in the cytosol bound to 14-3-3, a specific phosphoserine-binding protein.
The growth factors that promote cell survival activate the threonine kinase Akt which phosphorylates Bad causing suppression of apoptosis.[2] Substitution of the serine phosphorylation sites indicated that phosphorylation of Bad inactivated the molecule to promote cell survival.[4] Akt phosphorylates Bad *in vivo* and *in vitro* and blocks the Bad-induced death of primary neurons in a site specific manner.[5] Akt has not been shown to significantly phosphorylate Bad on ser112.

PKA-specific inhibitors block IL-3-induced phosphorylation on S112 of endogenous Bad and on mitochondria-based Bad S112 kinase activity. A blocking peptide that disrupts type II PKA holoenzyme association with A-kinase-anchoring proteins (AKAPs) also inhibited Bad phosphorylation and eliminated the Bad S112 kinase activity at mitochondria. Thus, the anchoring of PKA to mitochondria represents a focused subcellular kinase/substrate interaction that inactivates Bad at its target organelle in response to a survival factor.[6]

Reagents

Anti-phospho-Bad (Phosphoserine 112) is supplied as affinity isolated antibody in 0.07 M Tris-glycine, pH 7.4, containing 0.105M NaCl, 30% glycerol and 0.05% sodium azide.
Antibody concentration is approximately 0.7 mg/ml.

Precautions and Disclaimer

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

Storage/Stability

Store at 0 °C to –20 °C. 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

Recommended working concentration is 2 µg/ml using immunoprecipitated Bad from RIPA lysates of EGF stimulated A431 cells, anti-sheep IgG-peroxidase conjugate and a chemiluminescent detection system.

Note: Because of low endogenous levels of phospho-Bad , it is recommended to immunoprecipitate total Bad prior to immunoblotting.

Note: In order to obtain best results and assay sensitivities to different techniques and preparations, we recommend determining optimal working dilutions by titration test.

References

1. Farrow, S. N., and Brown, R. Curr. Opin. Genet. Dev., **6**, 45 (1996).
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3. Yang, E., et al., Cell, **80**, 285 (1995).
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