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ProductInformation

ANTI-PHOSPHO-BAD (PHOSPHOSERINE 136)
Developed in Sheep,
Affinity Isolated Antibody

Product Number **B 5804**

Product Description

Anti-phospho-Bad (Phosphoserine 136) is developed in sheep using a synthetic peptide [RGRSR(pS)APPNL] as immunogen. This sequence corresponds to amino acids 131-141 of mouse Bad. Whole sheep antiserum is purified using protein G and immunoaffinity chromatography to provide affinity isloated antibody.

Anti-phospho-Bad (Phosphoserine 136) recognizes mouse pS136 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.[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. Stimulation of the PI 3 kinase pathway results in activation of protein kinase B (PKB; also known as c-Akt and Rac) and is sequestered in the cytosol bound to 14-3-3, a specific phosphoserine-binding protein. A modest increase in intracellular Ca^{2+} concentration also promotes survival of some cultured neurons through a pathway that requires calmodulin but is independent of PI 3 kinase and the MAP kinases. Ca2+/calmodulin-dependent protein kinase kinase (CaM-KK) activates PKB directly, resulting in phosphorylation of BAD on serine residue 136 and the interaction of BAD with protein 14-3-3.[5]
In COS-7 cells, ceramide signals Raf-1 activation through Ras, but not apoptosis.[6] However, expression of small amounts Bad conferred ceramide-induced apoptosis onto COS-7 cells. Ceramide signaled apoptosis in Bad-expressing cells by a pathway involving sequentially kinase suppressor of Ras (KSR)/ceramide-activated protein kinase, Ras, c-Raf-1, and MEK1. Downstream, this pathway linked to Bad dephosphorylation at serine 136 by prolonged inactivation of Akt/PKB. Further, mutation of Bad at serine 136 abrogated ceramide signaling of apoptosis.[7]

Reagents

Anti-phospho-Bad (Phosphoserine 136) 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

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