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Phospho-Bad(S99) Antibody

Affinity Purified Rabbit Polyclonal Antibody (Pab)
Catalog # AP3039a

Product Information

Application	IF, DB, IHC-P, E
Primary Accession	Q92934
Other Accession	O35147, Q61337
Reactivity	Human
Predicted	Mouse, Rat, Bovine, Canine
Host	Rabbit
Clonality	Polyclonal
Isotype	Rabbit IgG
Calculated MW	18392

Additional Information

Gene ID	572
Other Names	Bcl2-associated agonist of cell death, BAD, Bcl-2-binding component 6, Bcl-2-like protein 8, Bcl2-L-8, Bcl-xL/Bcl-2-associated death promoter, Bcl2 antagonist of cell death, BAD, BBC6, BCL2L8
Target/Specificity	This Bad Antibody is generated from rabbits immunized with a KLH conjugated synthetic phosphopeptide corresponding to amino acid residues surrounding S99 of human Bad.
Dilution	IF~~1:200 DB~~1:500 IHC-P~~1:100~500 E~~Use at an assay dependent concentration.
Format	Purified polyclonal antibody supplied in PBS with 0.09% (W/V) sodium azide. This antibody is purified through a protein A column, followed by peptide affinity purification.
Storage	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
Precautions	Phospho-Bad(S99) Antibody is for research use only and not for use in diagnostic or therapeutic procedures.

Protein Information

Name	BAD
Synonyms	BBC6, BCL2L8

Function	Promotes cell death. Successfully competes for the binding to Bcl-X(L), Bcl-2 and Bcl-W, thereby affecting the level of heterodimerization of these proteins with BAX. Can reverse the death repressor activity of Bcl-X(L), but not that of Bcl-2 (By similarity). Appears to act as a link between growth factor receptor signaling and the apoptotic pathways.
Cellular Location	Mitochondrion outer membrane. Cytoplasm {ECO:0000250|UniProtKB:Q61337}. Note=Colocalizes with HIF3A in the cytoplasm (By similarity). Upon phosphorylation, locates to the cytoplasm. {ECO:0000250|UniProtKB:Q61337}
Tissue Location	Expressed in a wide variety of tissues.

Background

Bad is a member of the BCL-2 family. BCL-2 family members are known to be regulators of programmed cell death. This protein positively regulates cell apoptosis by forming heterodimers with BCL-xL and BCL-2, and reversing their death repressor activity. Proapoptotic activity of this protein is regulated through its phosphorylation. Protein kinases AKT and MAP kinase, as well as protein phosphatase calcineurin are found to be involved in the regulation of this protein. Bad is phosphorylated on one or more of Ser-75, Ser-99, Ser-118 and Ser-134 in response to survival stimuli, which blocks its pro-apoptotic activity. Phosphorylation on Ser-99 or Ser-75 promotes heterodimerization with 14-3-3 proteins. This interaction then facilitates the phosphorylation at Ser-118, a site within the BH3 motif, leading to the release of Bcl-X(L) and the promotion of cell survival. Ser-99 is the major site of AKT/PKB phosphorylation, Ser-118 the major site of protein kinase A (CAPK) phosphorylation.

References

References for protein:
1.Hurbin, A., et al., J. Biol. Chem. 280(20):19757-19767 (2005).
2.Antignani, A., et al., Biochemistry 44(10):4074-4082 (2005).
3.Ying, S., et al., Infect. Immun. 73(3):1399-1403 (2005).
4.Seo, S.Y., et al., J. Biol. Chem. 279(40):42240-42249 (2004).
5.Lee, J.W., et al., Carcinogenesis 25(8):1371-1376 (2004).

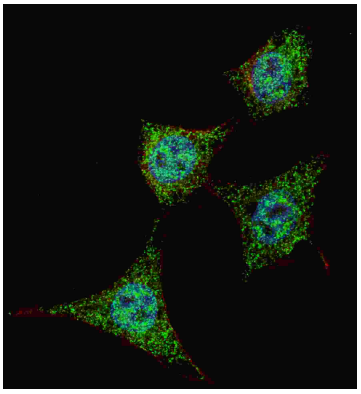
References for HeLa cell line:
1. Scherer WF, Syverton JT, Gey GO (May 1953). "Studies on the propagation in vitro of poliomyelitis viruses. IV. Viral multiplication in a stable strain of human malignant epithelial cells (strain HeLa) derived from an epidermoid carcinoma of the cervix". J. Exp. Med. 97 (5): 695–710. [PubMed:13052828].

2. Macville M, Schr□ck E, Padilla-Nash H, Keck C, Ghadimi BM, Zimonjic D, Popescu N, Ried T (January 1999). "Comprehensive and definitive molecular cytogenetic characterization of HeLa cells by spectral karyotyping". Cancer Res. 59 (1): 141–50. [PubMed: 9892199].
3. Rahbari R, Sheahan T, Modes V, Collier P, Macfarlane C, Badge RM (April 2009). "A novel L1 retrotransposon marker for HeLa cell line identification". BioTechniques 46 (4): 277–84. [PubMed: 19450234].

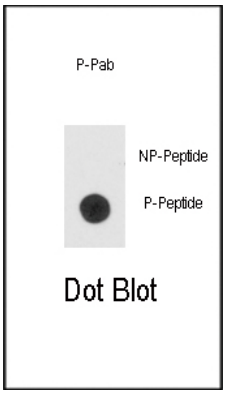
4. Capes-Davis A, Theodosopoulos G, Atkin I, Drexler HG, Kohara A, MacLeod RA, Masters JR, Nakamura Y, Reid YA, Reddel RR, Freshney RI (July 2010). "Check your cultures! A list of cross-contaminated or misidentified cell lines". Int. J. Cancer 127 (1): 1–8. [PubMed:20143388].

Images

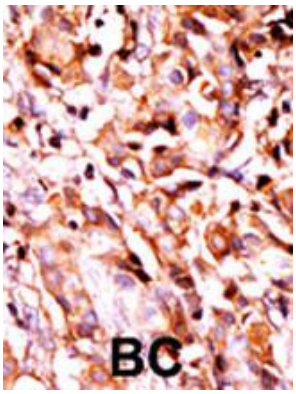
Fluorescent confocal image of HeLa cells stained with phospho-Bad-S99 antibody. HeLa cells were fixed with 4%



PFA (20 min), permeabilized with Triton X-100 (0.2%, 30 min). Cells were then incubated with AP3039a phospho-Bad-S99 primary antibody (1:200, 2 h at room temperature). For secondary antibody, Alexa Fluor® 488 conjugated donkey anti-rabbit antibody (green) was used (1:1000, 1h). Nuclei were counterstained with Hoechst 33342 (blue) (10 µg/ml, 5 min). Note the highly specific localization of the phospho-Bad-S99 mainly to the cytoplasm.



Dot blot analysis of anti-hBad-pS99 Phospho-specific Pab (Cat.#AP3039a) on nitrocellulose membrane. 50ng of Phospho-peptide or Non Phospho-peptide per dot were adsorbed. Antibody working concentrations are 0.5ug per ml.



Formalin-fixed and paraffin-embedded human cancer tissue reacted with the primary antibody, which was peroxidase-conjugated to the secondary antibody, followed by AEC staining. This data demonstrates the use of this antibody for immunohistochemistry; clinical relevance has not been evaluated. BC = breast carcinoma; HC = hepatocarcinoma.

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