3050 Spruce Street, Saint Louis, MO 63103 USA Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757 email: techservice@sial.com sigma-aldrich.com

Anti-Bad

produced in rabbit, affinity isolated antibody

Catalog Number B0684

Product Description

Anti-Bad is produced in rabbit using as immunogen Keyhole Limpet Hemocyanin (KLH) coupled synthetic peptide, MFQIPEFEPSEQEDSSAERGC (corresponding to amino acids 1-21 of human Bad). Cysteine is added to the C-terminus for coupling to an affinity matrix. Affinity isolated antibody is obtained from rabbit anti-Bad antiserum by immuno-specific purification which removes essentially all rabbit serum proteins, including immunoglobulins, which do not specifically bind to the peptide.

Anti-Bad recognizes human and mouse Bad by immunoblotting.

In multi-cell organisms the regulation of cell survival is crucial to normal physiology. This mechanism may be linked to excessive cell death or survival, which may play a role in a number of disease processes. The ratio of anti-(Bcl-2, Bcl-X_L, Mcl-1, and A1) to pro-(Bax, Bak, Bcl-Xs, and Bad) apoptotic molecules dictates whether a cell will respond to a proximal apoptotic stimulus. ^{2, 3}

Bad (Bcl-2-antagonist of cell death), initially identified by its interaction with Bcl-2 and Bcl- X_L , is a distant Bcl-2 family member. Bad 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 antiapoptotic effects of Bcl- X_L or Bcl-2.4 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 IL-3, a survival factor. 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. Substitution of the serine

phosphorylation sites indicated that phosphorylation of Bad inactivated the molecule to promote cell survival.⁵ Akt phosphorylates Bad *in vivo* and *in vitro* and blocks the Bad-induced death of primary neurons in a site specific manner.¹

Reagent

Supplied as a lyophilized powder from a 0.2 μ m filtered solution of phosphate buffered saline and 5% trehalose.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices

Preparation Instructions

To one vial of lyophilized powder, add 500 μ L of PBS for an antibody concentration of 0.2 mg/mL.

Storage/Stability

Prior to reconstitution, store at –20 °C. After reconstitution, freeze in working aliquots. Avoid repeated freezing and thawing. Do not store in frost-free freezer.

Product Profile

<u>Western Blot</u>: An antibody concentration of 1.0 μ g/mL is recommended.

Note: In order to obtain the best results in various techniques and preparations, we recommend determining optimal working dilutions by titration.

References

- 1. Datta, S. R., et al., Cell, 91, 231 (1997).
- 2. Farrow, S. N., and Brown, R., *Curr. Opin. Genet. Dev.*, **6**, 45 (1996).
- 3. Oltvai, Z. N., et al., Cell, 74, 609 (1993).
- 4. Yang, E., et al., Cell, 80, 285 (1995).
- 5. Zha, J., et al., Cell, 87, 619 (1996).

KCP,KAA,PHC,CY 03/21-1