

## Product Information

### Monoclonal Anti-Bad, clone 64103

produced in rat, purified immunoglobulin

Catalog Number **B0559**

#### Product Description

Monoclonal Anti-Mouse Bad (rat IgG2A isotype) is derived from a mouse hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a rat immunized with the N-terminal portion of recombinant mouse Bad. The antibody is purified from the IgG fraction of tissue culture supernatant using Protein G chromatography.

Monoclonal Anti-Mouse Bad recognizes mouse Bad by immunoblotting. It does not detect human Bad. The antibody will also detect a 32 kDa band that co-migrates with recombinant Bad on immunoblots.

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.<sup>1</sup> The ratio of anti-(Bcl-2, Bcl-X<sub>L</sub>, Mcl-1, and A1) to pro-(Bax, Bak, Bcl-X<sub>s</sub>, and Bad) apoptotic molecules dictates whether a cell will respond to a proximal apoptotic stimulus.<sup>2,3</sup>

Bad (Bcl-2-antagonist of cell death), initially identified by its interaction with Bcl-2 and Bcl-X<sub>L</sub>, 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 anti-apoptotic effects of Bcl-X<sub>L</sub> or Bcl-2.<sup>4</sup> 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.<sup>2</sup> Phosphorylated Bad does not bind Bcl-X<sub>L</sub> 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.<sup>3</sup> Substitution of the serine phosphorylation sites indicated that phosphorylation of Bad inactivated the molecule to promote cell survival.<sup>5</sup>

Akt phosphorylates Bad *in vivo* and *in vitro* and blocks the Bad-induced death of primary neurons in a site specific manner.<sup>1</sup>

#### Reagent

Supplied as a lyophilized powder from a 0.2 µm filtered solution of PBS, pH 7.4, with 5% trehalose.

#### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

#### Preparation Instructions

Reconstitute to desired concentration with PBS containing 0.02% sodium azide.

#### 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

**Immunoblotting:** a working antibody concentration of approximately 1 µg/ml is recommended using extracts from 2 x 10<sup>6</sup> mouse cytotoxic T cell line (CTLL2) or L929 fibroblast cells in an enhanced chemiluminescent (ECL) assay.

**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).

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