



## Product Information

### MONOCLONAL ANTI-BAX

#### CLONE 2D2

Purified Mouse Immunoglobulin

Product Number **B 8554**

#### Product Description

Monoclonal Anti-Bax (mouse IgG1 isotype) is derived from the 2D2 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a synthetic peptide corresponding to amino acids 3-16 of the human Bax sequence conjugated to KLH.<sup>1</sup> The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-Bax reacts specifically with human<sup>1-3</sup> and bovine<sup>4</sup> Bax protein, but not with mouse or rat Bax.<sup>1</sup> The epitope recognized by the antibody resides within amino acids 3-16 of the human Bax protein.<sup>1</sup> The antibody may be used for immunoblotting<sup>1-4</sup> (21 kDa, and possibly as a dimer at 42 kDa) and in immunoaffinity purification.<sup>4</sup>

Apoptosis is an active process of cell death that controls cell numbers in a variety of tissues during embryonic development and throughout adult life. The prototypic regulator of mammalian cell death is the protooncogene *bcl-2*. In both normal and neoplastic tissues and in experimental situations, expression or overexpression of the *bcl-2* gene appears to protect cells from death, by preventing or delaying apoptosis.<sup>5</sup> Other genes seem to be also important in controlling cell death. Candidates include *bcl-x*, *bad*, *bak* and *bax*, which have a significant homology to *bcl-2*. The *bcl-x* gene encodes two proteins: Bcl-x<sub>L</sub> (a 241 a.a. protein), which like Bcl-2, promotes cell survival, and Bcl-x<sub>S</sub> (deleted in 63 a.a.), a splice variant of Bcl-x<sub>L</sub> that antagonizes Bcl-2 function. On the other hand, Bad and Bax enhance apoptosis and inhibit the protective functions of Bcl-x<sub>L</sub> (and to a lesser extent of Bcl-2) and Bcl-2, respectively.<sup>6-10</sup> Bcl-2, Bcl-x<sub>L</sub>, and Bax, each contain a stretch of hydrophobic amino acids, approx. 20 residues in length, at their C-termini. There is little amino acid sequence conservation within these tails, but based on hydropathy plot analysis they are presumed to function in anchoring these proteins into organelle membranes.<sup>3</sup> Bcl-2 (a 26 kDa protein) has been localized to the nuclear membrane, endoplasmic reticulum, and the outer mitochondrial membranes. Bcl-x<sub>L</sub> (27 kDa) has been localized to the outer membrane of mitochondria. Bax (21 kDa) is an integral organelle membrane protein,

in particular in mitochondria. However, significant amounts of Bcl-x<sub>L</sub> and most of the Bax proteins are not membrane-associated and appear to be cytosolic, according to other reports.<sup>1</sup> Bax is associated with organelles or bound to organelles by Bcl-2 or a soluble protein found in the cytosol.<sup>3</sup> Formation of Bax homodimers promotes cell death, and this could be blocked by Bax heterodimerization with Bcl-2 or Bcl-x<sub>L</sub>. Although the relative ratio of Bax homodimers to heterodimers has been proposed to serve as a sensory switch to regulate cell death,<sup>1,2,6</sup> this interaction is promoted by the presence of nonionic detergents, which stimulate Bax dimer formation. Other hypotheses propose the formation of channels in mitochondrial outer membranes,<sup>11</sup> or the interaction of these members with the PTP pore to regulate the release of cytochrome c.<sup>12</sup> Cytochrome c in turn activates caspase-3 to cause cell death. Antibodies reacting specifically with Bax protein are useful tools in the study of the unique subcellular localization of Bax, and of the intracellular redistribution of this protein upon induction of apoptosis.

#### Reagents

Monoclonal Anti-Bax is supplied as a solution in 0.01 M phosphate buffered saline pH 7.4, containing 15 mM sodium azide as a preservative.

Antibody Concentration: Approx. 2 mg/ml.

#### Precautions and Disclaimer

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

#### Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. 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

A working concentration of 1-5 µg/ml is determined by immunoblotting, using cultured human breast adenocarcinoma MCF-7 cells, activated with dexamethasone.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

### References

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