

## Product Information

**Anti-Bcl-2 antibody, Mouse monoclonal**  
clone 10C4, purified from hybridoma cell culture

Product Number **B9804**

### Product Description

Anti-Bcl-2 antibody, Mouse monoclonal (mouse IgG1 isotype) is derived from the 10C4 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a synthetic peptide corresponding to amino acids 61-76 of the mouse Bcl-2 sequence conjugated to KLH.<sup>1</sup> The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Product Number ISO2.

Monoclonal Anti-Bcl-2 reacts specifically with mouse<sup>1,2</sup> and rat<sup>1</sup> Bcl-2 protein. The epitope recognized by the antibody resides within amino acids 61-76 of the mouse Bcl-2 protein.<sup>1</sup> The antibody may be used for immunoblotting<sup>1,2</sup> (a doublet at approx. 26 kDa, and possibly also an additional band at approx. 30 kDa).

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>3</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>4-8</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 hydrophathy plot analysis they are presumed to function in anchoring these proteins into organelle

membranes.<sup>9</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>9</sup> Formation of Bax homodimers promotes cell death, and this can 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>1,2,4</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>10</sup> or the interaction of these members with the PTP pore to regulate the release of cytochrome c.<sup>11</sup> Cytochrome c in turn activates caspase-3 to cause cell death. Antibodies reacting specifically with Bcl-2 protein are useful tools in the study of the unique subcellular localization of Bcl-2, and of the intracellular redistribution of this protein upon induction of apoptosis.

### Reagents

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

This product is 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.

### 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, or 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

Immunoblotting: a working concentration of 2-10 µg/ml is determined using cultured rat osteosarcoma ROS cells.

**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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