

Product Information

Monoclonal Anti-Bcl-2

Clone Bcl-2-100

Mouse Ascites Fluid

Product Number **B 3170**

Product Description

Monoclonal Anti-Bcl-2 (mouse IgG1 isotype) is derived from the Bcl-2-100 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. A synthetic peptide, corresponding to residues 41-54 of the Bcl-2 protein, conjugated to thyroglobulin was used as the immunogen.¹ 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-Bcl-2 recognizes an epitope located within the amino acid sequence GAAPAGIFSSQPG (residues 41-54) of the human Bcl-2 protein.¹ The product reacts with the Bcl-2 protein (26 kD), by immunoblotting,^{1,2} immunohistochemistry¹⁻⁵ (frozen,¹⁻⁵ formalin-fixed, paraffin embedded¹⁻⁴ and methacarn-fixed⁴ tissue sections), immunocytochemistry,^{1,3,6-8} electron microscopy³ and immunoprecipitation.⁶ The staining pattern of normal and reactive lymphoid tissues by the product has been reported; the Bcl-2 protein is labeled in the regions of non-proliferating lymphoid cells.¹ It is also detected in various cultured cell lines (e.g., MCF-7, HeLa). The antigen is localized within the mitochondria (intracytoplasmic), in both cytocentrifuged cell preparations and in tissue sections.^{1,3} Significant improvement in the quality and localization of staining of routinely fixed tissues is obtained by retrieval of the antigen by boiling (e.g., by a microwave) in the presence of 10 mM citrate buffer. The antibody reacts with human¹⁻⁸ Bcl-2, but not with mouse Bcl-2.^{1,2}

Apoptosis, also known as programmed cell death, is an active process of cell death which 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* that encodes a 26 kD protein. The *bcl-2* gene was first described in follicular B-cell lymphomas with the t(14;18) translocation.⁹ This translocation brings the *bcl-2* gene to the immunoglobulin heavy chain locus. The resultant fusion gene is deregulated and leads to overexpression of the mRNA and protein. The *bcl-2* protooncogene protein is detectable in neoplastic cells from cases of human lymphoma in which the 14;18

chromosomal translocation is present, but also in lymphomas that lack this chromosomal rearrangement and in normal lymphoid tissue.¹ In both normal and neoplastic tissues and in experimental situations, expression or overexpression of the *bcl-2* gene product appears to protect cells from death by preventing or delaying apoptosis. For instance, *bcl-2*-transfected hematopoietic cell lines, including myeloid lines, have a prolonged survival in growth factor-deprived media and a better *in vitro* resistance to stress.¹⁰ Thus, Bcl-2 protects factor-dependent hematopoietic cell lines from death resulting from withdrawal of growth factor and protects lymphoid cells from exposure to γ -irradiation, calcium ionophores, and corticosteroids. High amounts of Bcl-2 protein are found in lymphoid malignancies and in normal tissue affected by apoptotic processes, including bone marrow. Bcl-2 enhances the survival of central and peripheral neurons grown in culture in the absence of neurotrophic factors. Not all types of cell death can be prevented by overexpression of Bcl-2.

Other genes may also be important in controlling cell death during neural development. Candidates include *bcl-x* and *bax*, two other isolated genes with significant homology to *bcl-2*. The *bcl-x* gene encodes two proteins: Bcl-x_L, which, like Bcl-2, promotes cell survival, and Bcl-x_S, a splice variant of Bcl-x_L that antagonizes Bcl-2 function. Bax also appears to antagonize Bcl-2 action, since growth factor-dependent hematopoietic cell lines overexpressing both *Bcl-2* and *Bax* die upon withdrawal of growth factors. Based on cell subfractionation studies, the Bcl-2 protein has been localized to the inner mitochondrial membrane.¹¹ Observation of immunostained neurons and lymphocytes expressing Bcl-2 with a confocal microscope, revealed a punctate cytoplasmic immunostaining resembling that of Rhodamine 123, which specifically targets mitochondria. High expression of Bcl-2 was associated with a low complete remission rate after intensive chemotherapy and with a significantly shorter survival.^{1,2} Immunochemical detection of Bcl-2 protein may help to distinguish between reactive and neoplastic lymphoid follicles.

Monoclonal Anti-Bcl-2 may be used for the localization of Bcl-2, using various immunochemical assays including immunoblotting, immunohistology, electron microscopy and immunoprecipitation.

Reagents

The product is provided as ascites fluid with 15 mM sodium azide as a preservative.

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 hazardous and safe handling practice.

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.

Product Profile

A minimum working dilution of 1:1,000 is determined by indirect immunoblotting using a HeLa cell extract.

In order to obtain best results, it is recommended that each user determine the optimal working dilutions for individual applications by titration assay.

References

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JWM/KMR 05/03

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