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ProductInformation

Monoclonal Anti-Bcl-2α, Mouse/Rat
Clone 10C4
Purified Mouse Immunoglobulin

Product Number **B 1809**

Product Description

Monoclonal Anti-Bcl-2α (mouse IgG1 isotype) is derived from the 10C4 hybridoma produced by the fusion of mouse myeloma NS cells and splenocytes from BALB/c mouse immunized with a synthetic peptide corresponding to amino acids 61-76 (Cys-VHREMAA-RTSPLRPLV) of mouse Bcl-2. The mouse ascites is purified by protein G affinity chromatography.[1]

Monoclonal Anti-Bcl-2α recognizes specifically a 25-26 kDa oncoprotein identified as mouse Bcl-2α. It crossreacts with rat Bcl-2α. It does not recognize human Bcl-2α. The product is useful in ELISA, immunoprecipitation immunoblotting[1], and immunohistochemistry (frozen and formalin-fixed paraffin-embedded tissue) applications.

Cell death due to apoptosis is executed by proteases called caspases. The Bcl family of intracellular proteins is the central regulator of caspase activation. Bcl-2α and Bcl-2β proteins are encoded by 5.5- and 3.5-kb mRNAs respectively and are identical except at their carboxyl termini.[2] The twenty known members of Bcl-2 family share at least one of the Bcl-2 homology domains 1, 2, 3, and 4 (BH1, BH2, BH3, and BH4). Members of the Bcl-2 family interact with each other via these BH domains. Some members of this family, like Bcl-2 itself and Bcl-x_L, Bcl-w, A1 and Mcl1, inhibit apoptosis, at least partly by blocking the release of cytochrome c from mitochondria. Other members of the Bcl family, including Bax, Bak, Bcl-x_S, Bid, Bad, Bik, promote apoptosis.[3-5]

Bcl-2 is located in the outer mitochondrial membrane, endoplasmic reticulum, or nuclear membrane. Mutations causing excessive production of Bcl-2 inhibit cell death and promote development of cancer in B-lymphocytes, which acquire unlimited capacity for self-renewal.[5,6] Bcl-2 apoptosis suppressor activity may be dependent on its phosphorylation state as shown in the experiments with PC-3 cells treated with TNFα. Treatment resulted in the induction of insulin-like growth factor binding protein-3 (IGFBP-3) expression and induced apoptosis. When the IGFBP-3 was rendered inactive by the treatment with neutralizing antibodies, the apoptosis was prevented due to increased levels of the inactive, serine phosphorylated form of the survival protein Bcl-2.[7]

Reagent

Monoclonal anti-Bcl-2α is supplied as a solution at approximately 0.2 mg/ml antibody in phosphate buffered saline, pH 7.4.containing 0.2% bovine serum albumin and 15 mM sodium azide

Precautions and Disclaimer

Due to the sodium azide content, a material safety data 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 practices.

Storage/Stability

For prolonged storage, freeze in working aliquots at –70 °C. Repeated freezing and thawing is not recommended. Storage in “frost-free” freezers is also 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 2 to 4 µg/ml is determined by immunohistochemistry using mouse or rat thymocytes, splenocytes, tonsil, or follicular lymphomas. Staining of formalin-fixed tissue sections requires boiling in 10 mM citrate buffer, pH 6.0 for 10 to 20 minutes followed by cooling to room temperature for 20 minutes. For immunoblotting, the recommended dilution is 1:200 to 1:400, and 10 µl of antibody per 1 mg of protein lysate is suggested for immuno-precipitation.

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

References

1. Hsu, Y-T and Youle, R.J. Nonionic detergents induce dimerization among members of the Bcl-2 family. J. Biol. Chem., **272**, 13829-13874 (1997).
2. Tsujimoto, Y., Croce, C. M., Analysis of the structure, transcripts, and protein products of bcl-2, the gene involved in human follicular lymphoma. Proc. Nat. Acad. Sci. 83, 5214-5218 (1986).
3. Shi, Y. Mechanisms of caspase activation and inhibition during apoptosis. Mol. Cell, **9**, 459-470 (2002).
4. Cory, S., Adams, J.M., The Bcl2 family: regulators of the cellular life-or-death switch. Nature Reviews Cancer, **2**, 647-656 (2002).
5. Cory, S., Adams, J.M. The Bcl-2 protein family: arbiters of cell survival. Science, **281**, 1322-1326 (1998).
6. Reed, J.C., et al., Structure-function analysis of Bcl-2 family proteins. Regulators of programmed cell death. Adv. Exp. Med. Biol., **406**, 99-112 (1996).
7. Rajah, R., et al., Insulin-like growth factor binding protein-3 mediates tumor necrosis factor-α-induced apoptosis: role of Bcl-2 phosphorylation. Cell Growth Differ., **4**, 163-171 (2002).

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