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ProductInformation

MONOCLONAL ANTI-HUMAN FAS (CD95/APO-1), CLONE DX2
FITC Conjugate

Product Number **F 4229**

Product Description

Monoclonal Anti-Human Fas (CD95/Apo-1) (mouse IgG1 isotype) is derived from the DX2 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a C3H mouse immunized with murine L cells transfected by a human Fas/CD95 cDNA.[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). The antibody is purified from culture supernatant of the hybridoma cells grown in a bioreactor, and conjugated with fluorescein isothiocyanate isomer I. The conjugate is purified by gel filtration and contains no detectable free FITC.

Monoclonal Anti-Human Fas (CD95/Apo-1) - FITC Conjugate, reacts specifically with the functional epitope of the human Fas (CD95/Apo-1) antigen.[1] The antibody is reactive in flow cytometry[1-5] and in the induction of apoptosis.[1]

Homeostasis of multicellular organisms is controlled not only by the proliferation and differentiation of cells but also by cell death. The death of cells during embryogenesis, metamorphosis, endocrine-dependent tissue atrophy, a variety of pathologic conditions, and normal tissue turnover, is called programmed cell death (PCD). Most of PCD proceeds by apoptosis, a process that includes condensation and segmentation of nuclei, condensation and fragmentation of the cytoplasm, and often extensive fragmentation of chromosomal DNA into nucleosome units.[6] Many cells can be activated to undergo apoptosis following the interaction of selected ligands with cell surface receptors. The most studied receptors are CD95/Fas/Apo-1 (apoptosis inducing protein 1) and tumor necrosis factor receptor 1 (TNFR1). Apoptosis mediated by both signaling cascades results in activation of a family of cystein proteases known as caspases. However, Fas-mediated death occurs much more rapidly than that triggered by the TNFR1. Engagement of Fas by its ligand (Fas ligand, FasL, CD95L), or by an appropriate antibody, results in the rapid induction of PCD in susceptible cell lines. This process bypasses the usual long sequence of signaling enzymes and immediately activates preexisting caspases.[7] The action of Fas is mediated via FADD (Fas-associated death domain)/ MORT1, an adapter protein that has a death domain at its C-terminus and binds to the cytoplasmic death domain of Fas. Human CD95/Fas/Apo-1 antigen is a 325 amino acids single transmembrane glycoprotein receptor (45-48 kDa).[8,9] Primary sequence analysis of the extracellular portion of CD95/Fas/Apo-1 has revealed strong homologies with the extracellular domain of receptors belonging to the TNF receptor family, which includes TNF receptor types 1 and 2 (TNFR1/2), the low affinity nerve growth factor receptor, and lymphocyte receptors such as CD27, CD30, CD40, and OX40.[8,9] An integral membrane protein, with strong homology to TNF-α and -β, has been identified as Fas ligand.[1] A moderate degree of homology (26% identity in a stretch of 65 amino acids) between the intracellular portion of the human CD95 and the 55 kDa TNFR1, has been observed. Mutational analysis of this domain has revealed its involvement in the generation of the apoptotic signal from both CD95 and TNFR1.[1] Thus, a common effector may transduce the apoptotic signal from both receptors. The cellular pathways that control apoptosis are critical to the maturation, selection, and survival of lymphocytes. Apoptosis or cell suicide is the physiological mode of lymphoid cell death in circumstances like negative selection of T cells in the thymus, ligation of CD4 and CD3 in mature T cells, downregulation of the immune response, clonal deletion of B cells by antigen, death of killer cell targets, cytokine-mediated killing, and tumor regression. Fas is expressed on a number of lymphoma cell lines, on Epstein-Barr virus-transformed B lymphoblasts, and on a proportion of activated B and T cells. Upon contact with an anti-Fas antibody, some lymphocytes expressing Fas antigen undergo apoptosis.[10,11] Fas has also

been detected in soluble form and this form of the protein is thought to play a role in regulating certain aspects of immune system function. Elevated levels of soluble Fas have been detected in sera from patients with leukemic diseases, as well as in patients with systemic lupus erythematosus. Therefore, altered levels of secreted Fas protein is likely to be involved in the abnormal growth regulation of lymphoid cells. The production of excess soluble Fas protein would prevent cells from undergoing Fas ligand induced apoptosis and thereby permit tumor cells to escape immunosurveillance. Antibodies reacting specifically with CD95 (Fas, Apo-1) are useful tools in the study of the intracellular pathways leading from membrane receptor engagement to apoptotic cell death, the tissue distribution and developmental expression pattern of Fas, and its essential role during mammalian development especially in immune system homeostasis.

Reagents
The conjugate is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15 mM sodium azide as a preservative.

Specific Antibody Concentration 1-2 mg/ml.
The F/P molar ratio of the product is in the range of 3-8.

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 practices.

Storage/Stability
For continuous use and extended storage, store at 2-8 °C. Protect from prolonged exposure to light. 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 minimum working diluton of 1:50 is determined by flow cytometry, using cultured human Burkitt's lymphoma Raji cells.

When assayed by flow cytometric analysis (with a FACScan flow cytometer), using 10 µl of antibody at working dilution, to stain $1x10^6$ cells/0.1ml/test, fluorescence intensity is observed similar to that obtained with saturating antibody levels. The percentage population positive is also at the maximum percentage positive, using saturating antibody levels.

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

References

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