DakoCytomation

Monoclonal Mouse
Anti-Human BCL2 Oncoprotein/FITC
Clone 124
Code No. F 7053

For research use only. Not for use in diagnostic procedures.

Recommended use

Monoclonal Mouse Anti-Human BCL2 Oncoprotein/FITC, is recommended for use in flow cytometry for identification of cells expressing the BCL2 protein.

Introduction

The BCL2 protein is encoded by the *BCL2* gene involved in the t(14;18) chromosomal translocation (1). This cytogenetic abnormality brings the *BCL2* gene into juxtaposition with the immunoglobulin heavy chain gene and causes overexpression of BCL2 protein (2). The 14;18 chromosomal translocation is not a prerequisite for BCL2 protein expression since this occurs in many cases without this rearrangement (1). The BCL2 (molecular mass 26 kDa) is an integral membrane protein which lies within the cell rather than on the cell surface (3, 4). The protein is associated with mitochondria, smooth endoplasmic reticulum and perinuclear membrane and plays a central role in the inhibition of apoptosis (programmed cell death) (3-6). A review on the growing BCL2 family has been published (7). Another review deals with BCL2 detection in haematopoietic and non-haematopoietic tissues (8).

Reagent provided

Purified monoclonal mouse antibody conjugated with fluorescein isothiocyanate isomer 1 (FITC). The conjugate is provided in liquid form in buffer containing 1% bovine serum albumin (BSA) and 15 mmol/L NaN_3, pH 7.2. Each vial contains 100 tests (10 µL of conjugate for up to 10^5 leucocytes from normal human peripheral blood).

Clone: 124 (1). Isotype: IgG1, kappa. Conjugate concentration mg/L: See label on vial.

Immunogen

Synthetic peptide comprising amino acids 41-54 of human BCL2 protein (1, 9).

Specificity

The DakoCytomation antibody reacts specifically with BCL2 oncoprotein as demonstrated by immunoblotting and immunoprecipitation (1).

In lymphoid tissue, the antibody reacts with small B lymphocytes in the mantle zone and many cells within T cell areas in lymphoid tissue (1). Very few cells in germinal centres are stained (1). In the thymus many cells in the medulla are stained, but in the cortex weak or negative staining of most cells is seen (1). In non-haematopoietic tissue few cells are stained (principally representing infiltrating leucocytes) (1).

Flow cytometric studies revealed that more than 80 % of T and B cells are BCL2-positive. After mitogen stimulation in vitro the BCL2 reactivity decreased slightly in T cells, but markedly in B cells. It was also shown that the BCL2 oncoprotein expression was not restricted to a specific phase in the cell cycle (10).

Precautions

1. The device is not intended for clinical use including diagnosis, prognosis, and monitoring of a disease state, and it must not be used in conjunction with patient records or treatment.

2. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.

3. As with any product derived from biological sources, proper handling procedures should be used.

Storage

Store in the dark at 2-8 °C. Do not use after expiration date stamped on vial. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our Technical Services.

Staining Procedure

1. Transfer 50 µL cell suspension to be analysed to each of two test tubes, labelled 1 and 2 (test and control).
2. Add 100 µL DakoCytomation IntraStain Reagent A (Fixation) to each tube. Vortex gently.
3. Incubate at room temperature for 15 minutes.
4. Add 2 mL PBS and mix well.
5. Centrifuge at 300 x g for 5 minutes, then aspirate the supernatant, leaving approximately 50 µL of fluid.
6. Add 100 µL DakoCytomation IntraStain Reagent B (Permeabilisation) to each tube. To test tube 1, add 10 µL F 7053. To test tube 2, add 10 µL of a suitably matched, FITC-conjugated negative control, e.g. Code No. X 0927. Vortex each tube gently to ensure that the cells are in suspension.
7. Incubate in the dark at room temperature for 15 minutes.
8. Repeat steps 4 and 5.
9. Resuspend the pellet in an appropriate fluid for flow cytometric analysis, such as 1% paraformaldehyde in PBS.
10. Analyse on a flow cytometer

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It is recommended to include a suitable positive and negative control sample with each run for reagent and preparation control. Note that fluorochrome conjugates are light sensitive, and samples should be protected from light during the staining procedure and until the analysis.

References

1. Pezzella F, Tse AGD, Cordell JL, Pulford KAF, Gatter KC, Mason DY. Expression of the bcl-2 oncogene protein is not specific for the 14;18 chromosomal translocation. Am J Pathol 1990;137:225-32.
2. Cleary ML, Smith SD, Sklar J. Cloning and structural analysis of cDNAs for bcl-2 and a hybrid bcl-2/immunoglobulin transcript resulting from the t(14;18) translocation. Cell 1986;47:19-28.
3. Hockenbery D, Nuñez G, Milliman C, Schreiber RD, Korsmeyer SJ. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature 1990;348:334-6.
4. Tsujimoto Y, Croce CM. Analysis of the structure, transcripts, and protein products of bcl-2, the gene involved in human follicular lymphoma. Proc Natl Acad Sci (USA) 1986;83:5214-8.
5. Krajewski S, Tanaka S, Takayama S, Schibler MJ, Fenton W, Reed JC. Investigation of the subcellular distribution of the bcl-2 oncoprotein: Residens in the nuclear envelope endoplasmatic reticulum and outer mitochondrial membranes. Cancer Res 1993;53:4701-14.
6. Korsmeyer SJ. Bcl-2 initiates a new category of oncogenes: regulators of cell death. Blood 1992;80:879-86.
7. Cory S, Adams JM. The BCL2 family: regulators of the cellular life-or-death switch. Nature Reviews 2002;2:647-56..
8. Pezzella F, Gatter K. What is the value of bcl-2 detection for histopathologists? Histopathology 1995;26:89-93.
9. Liu YJ, Mason DY, Johnson GD, Abbot S, Gregory CD, Hardie DL, et al. Germinal center cells express bcl-2 protein after activation by signals which prevent their entry into apoptosis. Eur J Immunol 1991;21:1905-10.
10. Aiello A, Delia D, Borrello MG, Biassoni D, Giardini R, Fontanella E, et al. Flow cytometric detection of the mitochondrial BCL-2 protein in normal and neoplastic human lymphoid cells. Cytometry 1992;13:502-9.

Explanation of symbols

REF	Catalogue number		Keep away from sunlight (consult storage section)		Manufacturer
	Consult instructions for use	LOT	Batch code		
2 °C 8 °C	Temperature limitation		Use by		

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