

Analyte Specific Reagent.

Analytical and performance characteristics are not established.

SPECIFICITY

The CD40 antigen is a 44-48 kDa type I integral membrane protein of the tumor necrosis factor receptor (TNFR) superfamily (1, 2). This antigen is found on B cell lines, is strongly expressed by interdigitating cells (IDC), basal epithelial cells, and is also present on macrophages, some endothelial cells, and follicular dendritic cells. It is a pan-B marker, absent only from terminally differentiated plasma cells (2).

CD40 is implicated in the process of B cell selection in the germinal centre. Studies demonstrated that CD40 monoclonal antibodies (mAbs) induce strong homotypic adhesions in resting B cells and, together with interleukin-4 (IL-4) maintain the cell cycle of blasts of the B lineage. They also can promote the switch to IgE secretion (3). Activated B cells via CD40 antigen in the presence of IL-10 differentiate into plasma cells and secrete large amounts of immunoglobulins (4). The CD154, (CD40 ligand) is a membrane glycoprotein on activated T cells that induces B cell proliferation and immunoglobulin secretion (5, 6). CD154 is also expressed on activated platelets and triggers an inflammatory reaction of endothelial cells (7).

The MAB89 mAb reacts specifically with CD40 (8).

The MAB89 mAb was assigned to the CD40 cluster of differentiation at the 6th International Workshop on Human Leucocyte Differentiation Antigens in Kobe, Japan, in 1996 (2).

REAGENT

IOTest CD40-PE Conjugated Antibody

PN IM1936U – 2 mL Liquid – 20 µL / test*.

Clone MAB89

Isotype IgG1, mouse

Immunogen Anti-µ activated tonsillar human B cells

Hybridoma NS1 X Balb/c

Source Ascites fluid

Purification Ion exchange or affinity chromatography

Conjugation R-phycoerythrin (PE) is conjugated at 0.5 – 1.5 moles of PE per mole of Ig.

Fluorescence PE (orange-red)
Excites at 486 – 580 nm
Emits at 568 – 590 nm

REAGENT CONTENTS

This reagent is provided in phosphate-buffered saline, with 0.1% sodium azide (NaN₃) as preservative, and 2.0 mg / mL bovine serum albumin (BSA).

STATEMENT OF WARNINGS

1. This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an

extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.

2. Do not use antibody beyond the expiration date on the label.
3. Samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
4. Never pipet by mouth and avoid contact of samples with skin and mucous membranes
5. Minimize exposure of reagent to light during storage or incubation.
6. Avoid microbial contamination of reagents or incorrect results might occur.
7. Use good laboratory practices when handling this reagent.

STORAGE CONDITIONS AND STABILITY

This reagent is stable up to the expiration date when stored at 2 – 8°C. Do not freeze. Minimize exposure to light.

EVIDENCE OF DETERIORATION

Any change in the physical appearance of this PE-labeled reagent (clear colorless to pinkish liquid) or any major variation in values obtained for control samples may indicate deterioration and the reagent should not be used.

REAGENT PREPARATION

No preparation is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 18 – 25°C prior to use.

SELECTED RESEARCH REFERENCES

1. Katira, A., Holder, M., Pound, J., Gordon, J. "CD40 workshop panel report", 1995, in *Leucocyte Typing V*, Schlossman, S.F., et al Eds, Oxford University Press, 547-550.
2. Challa, A., Holder, M., Baker, M., Pound, J., Gordon, J., "CD40 Workshop Panel report", 1997, *Leucocyte Typing VI*, White Cell Differentiation Antigens. Kishimoto, T., et al., Eds., Garland Publishing, Inc., 159-161.
3. Rousset, F., Garcia, E., Banchereau, J., "Cytokine-induced proliferation and immunoglobulin production of human B lymphocytes triggered through their CD40 Antigen", 1991, *J. Exp. Med.*, 173, 705-710.
4. Rousset, F., Garcia, E., Defrance, T., Péronne, C., Vezzio, N., Hsu, D.H., Kastelein, R., Moore, K.W., Banchereau J., "Interleukin 10 is a potent growth and

differentiation factor for activated human B lymphocytes", 1992, *Proc. Natl. Acad. Sci. USA.*, 89, 1890-1893.

5. Lane, P., Traunecker, A., Hubele, S., Inui, S., Lanzavecchia, A., Gray, D., "Activated human T cells express a ligand for the human B cell-associated antigen CD40 which participates in T cell-dependent activation of B lymphocytes", 1992, *Eur. J. Immunol.*, 22, 2573-2578.
6. Brugnoli, D., Airo, P., Graf, D., Marconi, M., Molinari, C., Braga, D., Malacarne, F., Soresina, A., Ugazio, A.G., Cattaneo, R., Kroczeck, R.A., Notarangelo, L.D., "Ontogeny of CD40 expression by activated peripheral blood lymphocytes in humans", 1996, *Immunol. Letters*, 49, 27-30.
7. Henn, V., Slupsky, J.R., Gräfes, M., Anagnostopoulos, I., Förster, R., Müller-Bergahaus, G., Kroczeck, R.A., "CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells", 1998, *Nature*, 391, 591-594.
8. Vallé, A., Zuber, C.E., Defrance, T., Djossou, O., De Rie, M., Banchereau, J., "Activation on human B lymphocytes through CD40 and interleukin 4", 1989, *Eur. J. Immunol.*, 19, 1463-1467.

PRODUCT AVAILABILITY

IOTest CD40-PE Conjugated Antibody
PN IM1936U – 2 mL Liquid – 20 µL / test*.

PE is licensed under patent 4,520,110.

For additional information in the USA, call 800-526-7694.

Outside the USA, contact your local Beckman Coulter representative.

www.beckmancoulter.com

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(*) : 20 µL is the quantity of product sufficient to stain

5 x 10⁵ cells in a standard immunofluorescence assay