

MONOCLONAL ANTIBODY CD95 Fas

Cat. No.	Form	Concentration	Presentation
1504	Purified	500 µg/mL	Liquid 100 µL

Clone CH-11

Isotype IgM (mouse)

Immunogen FS-7 (Human diploid fibroblast cell line)

Hybridoma Myeloma (NS-1) x Mouse Balb/c spleen cells

Specificity

The CD95 (Fas or APO-1) antigen is a 40-50 kDa transmembrane glycoprotein. It belongs to the Nerve Growth Factor Receptor / Tumor Necrosis Factor (NGFR / TNF) superfamily and contains three cysteine-rich repeats. This cell surface molecule mediates apoptosis (programmed cell death) (1). CD95 antigen is expressed on a substantial proportion of peripheral CD4⁺ cells, CD8⁺ cells and B cells but on a minor proportion of Natural Killer (NK) cells. It is also expressed variably on granulocytes and monocytes (2). CD95 is strongly up-regulated on activated T cells, B cells, NK cells and thymocytes (3, 4). Moreover it is widely expressed on cell lines of T, B, NK and myeloid lineage.

CH-11 antibody specifically reacts with human Fas (CD95) but not with mouse Fas (1-5). This antibody induces apoptosis of some CD95 expressing cell lines, in vitro (1, 4-7).

CH-11 antibody ("Anti-Fas") has been assigned to the CD95 cluster of differentiation at the Vth International Workshop on Human Leucocyte Differentiation Antigens in Boston, in 1993 (8).

Applications

In vitro induction of apoptosis of CD95-expressing cells
 Flow cytometry
 Immunohistochemistry (frozen tissue sections)
 Immunoblotting

Buffer

PBS / glycerol, no added preservative.

Storage

The purified liquid form should be stored at -20°C until the expiration date stated on the vial label.

October 8, 1996

MA001

FOR RESEARCH USE ONLY - NOT FOR USE IN DIAGNOSTIC PROCEDURES



IMMUNOTECH
A COULTER COMPANY

B.P. 177 - 13276 Marseille Cedex 9 France
 Tel. (33) 91 17 27 00 - Fax. (33) 91 41 43 58

Recommended ProceduresInduction of apoptosis

Generally, after a 16 hour-incubation at 37°C (5 % CO₂), this antibody, at the concentration of 0.1 to 1 µg/mL, induces apoptosis in most sensitive CD95-expressing cell cultures at the density of 5 x 10⁴ cells/mL.

Note:

The apoptosis activity may vary from lot to lot. Please refer to the enclosed performance data sheet corresponding to the current lot.

Flow cytometry

4 µL/5 x 10⁵ cells/test or 100 µL whole blood.

Immunohistochemistry

Working concentration: 20 µg/mL

This antibody is only suitable for frozen tissue sections.

Immunoblotting

Working concentration: 20 µg/mL

References

- 1) Itoh, N., Yonehara, S., Ishii, A., Yonehara, M., Mizushima, S.-I., Sameshima, M., Hase, A., Seto, Y., Nagata, S., "The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis", 1991, Cell, **66**, 233-243.
- 2) Miyawaki, T., Uehara, T., Nibu, R., Tsuji, T., Yachie, A., Yonehara, S., Taniguchi, N., "Differential expression of apoptosis-related Fas antigen on lymphocyte subpopulations in human peripheral blood", 1992, J. Immunol., **149**, 3753-3758.
- 3) Yoshino, T., Kondo, E., Cao, L., Takahashi, K., Hayashi, K., Nomura, S., Akagi, T., "Inverse expression of bcl-2 protein and Fas antigen in lymphoblasts in peripheral lymph nodes and activated peripheral blood T and B lymphocytes", 1994, Blood, **83**, 1856-1861.
- 4) Yonehara, S., Nishimura, Y., Kishii, S., Yonehara, M., Takazawa, K., Tamatani, T., Ishii, A., "Involvement of apoptosis antigen Fas in clonal deletion of human thymocytes", 1994, Int. Immunol., **6**, 1849-1856.
- 5) Yonehara, S., Ishii, A., Yonehara, M., "A cell-killing monoclonal antibody (Anti-Fas) to a cell surface antigen co-downregulated with the receptor of tumor necrosis factor", 1989, J. Exp. Med., **169**, 1747-1756.
- 6) Kobayashi, N., Hamamoto, Y., Yamamoto, N., Ishii, A., Yonehara, M., Yonehara, S., "Anti-Fas monoclonal antibody is cytotoxic to human immunodeficiency virus-infected cells without augmenting viral replication", 1990, Proc. Natl. Acad. Sci., **87**, 9620-9624.
- 7) Mollereau, B., Deckert, M., Déas, O., Rieux-Laucat, F., Hirsch, F., Bernard, A., Fischer, A., Lynch, D.H., Charpentier B., Le Deist, F., Senik, A., "CD2-induced apoptosis in activated human peripheral T cells", 1996, J. Immunol., **156**, 3184-3190.
- 8) Robertson, M.J., Ritz, J., "Cluster report: CD95", 1995, in Leucocyte Typing V, Schlossman, S.F., et al., Eds., Oxford University Press, 1142-1143.

CONTROL DATA SHEET
Cytotoxicity assay

Anti-Fas (CD95), Clone CH-11, Cat N°1504

Lot Number:150

Culture and test conditions

WR19L-12a cells (mouse T cells transfected with human Fas cDNA, 1) were plated on poly-L-lysine-coated 96-well microtiterplate, and cultured overnight in DMEM 10% FCS at 37°C (5% CO₂) at 2500 cells/well in 50µL medium.



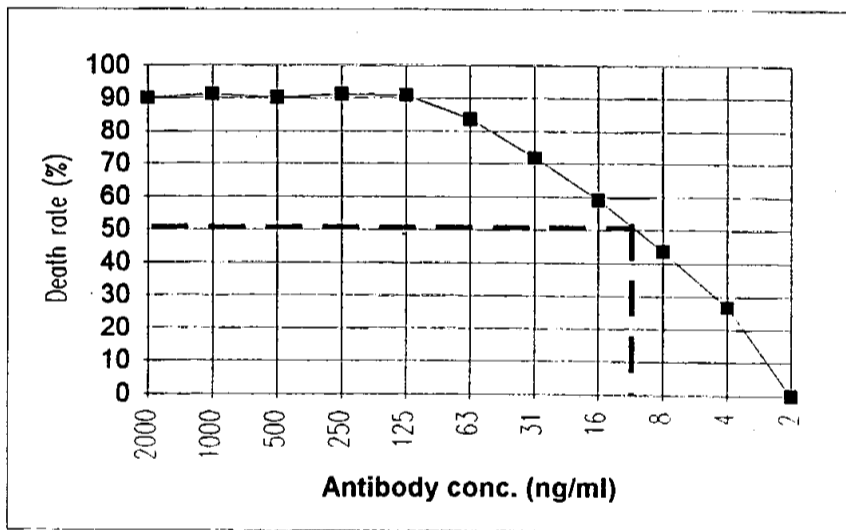
50µL of 4000 ~3.9ng/mL anti-Fas diluted in DMEM 10% FCS was added to wells (final concentration: 2000 ~1.95ng/mL) and cultured for 16 hours at 37°C (5% CO₂).



Cell viability was calculated by MTT assay (1).

Results

Antibody conc. (50% death): 8-16ng/mL .



Reference: 1) Itoh, N. et al. (1991), Cell, 66, 233-243.

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