Technical Data Sheet

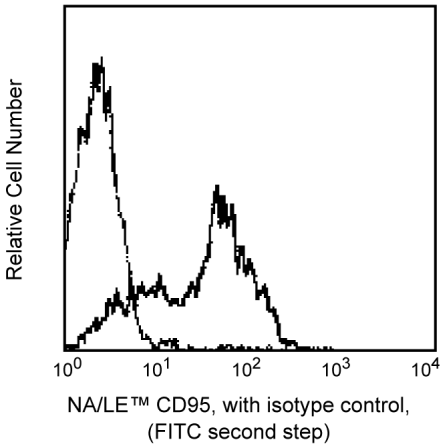
Purified NA/LE Mouse Anti-Human CD95

Product Information

Material Number:	**555670**
Alternate Name:	APO-1; FAS; TNFRSF6; APT1; ALPS1A; FAS1; FASTM; FASLG receptor
Size:	0.5 mg
Concentration:	1.0 mg/ml
Clone:	DX2
Immunogen:	Human CD95-transfected L Cells
Isotype:	Mouse (C3H) IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Rhesus, Cynomolgus, Baboon
Workshop:	VI C-64
Storage Buffer:	No azide/low endotoxin: Aqueous buffered solution containing no preservative, 0.2µm sterile filtered. Endotoxin level is ≤0.01 EU/µg (≤0.001 ng/µg) of protein as determined by the LAL assay.

Description

The DX2 monoclonal antibody specifically binds to the human Fas antigen (also called APO-1). This 45 kDa type I transmembrane glycoprotein was designated as CD95 at the Fifth HLDA Workshop. Fas is a member of the TNF-receptor superfamily and is also known as Tumor necrosis factor receptor superfamily member 6 (TNFRSF6). It is differentially expressed on a variety of normal and neoplastic cells. These include some undifferentiated thymocytes, and activated T and B lymphocytes, natural killer (NK) cells, monocytes, neutrophils, fibroblasts, and cell lines. CD95 is preferentially expressed on CD45RO-positive memory T lymphocytes and γ/δ T lymphocytes. The Fas/CD95 antigen is a polypeptide that plays a role in the programmed sequence of events leading to cell death, termed apoptosis. Crosslinking CD95 with DX2 antibody delivers an apoptotic signal indicating that DX2 recognizes a functional epitope of the CD95 antigen.



Flow cytometric analysis of CD95 expression on human peripheral blood lymphocytes. *Whole blood was stained with either Purified NA/LE Mouse Anti-Human CD95 (Cat. No. 555670; solid line histogram) or Purified NA/LE Mouse IgG1 κ Isotype Control (Cat. No. 554721; dashed line histogram), followed by FITC Goat Anti-Mouse IgG/IgM (Cat. No. 555988). Erythrocytes were lysed with BD Pharm Lyse™ Lysing Buffer (Cat. No. 555899).*

Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

This preparation contains no preservatives, thus it should be handled under aseptic conditions.

Application Notes

Application

Flow cytometry	Routinely Tested
Functional assay	Tested During Development

BD Biosciences

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Recommended Assay Procedure:
Investigators are advised that the following procedure is not routinely tested for this material.

Induction of Apoptosis Using Purified Mouse Anti-Human CD95 (Clone DX2) [MN 555670]
Additional Materials Needed:
Positive control cell line (e.g., Daudi, HPB-ALL, Jurkat)
Recombinant Protein G (rProt G) (SIGMA, Cat. No. P4689)
96-well microtiter plate
IMDM or RPMI 1640 medium with 10% heat-inactivated fetal bovine serum (FBS), 1% L-glutamine, 1% antibiotics (FBS medium)

Procedure:
1. Maintain cells in culture with 10% FBS medium; change the medium one day before starting the induction of apoptosis. Harvest and pellet the cells, resuspend in 10% FBS medium at a density of 0.5-1.0 x 10^6 cells/ml.

2. In a 96-well microtiter plate, add 2.5 µg/50 µl CD95 NA/LE™, 0.5 µg/50 µl rProt G, 0.5-1.0 x 10^5 cells and 10% FBS medium to a total volume of 200 µl. Negative controls should consist of: 1) 0.5-1.0 x 10^5 cells with 10% FBS medium alone (200 µl total volume), and 2) 0.5-1.0 x 10^5 cells with 0.5 µg/50 µl rProtG and 10% FBS medium.

3. Incubate the 96-well plate at 37°C, 5% CO2, for 12-24 hours. Traditionally, apoptosis has been observed by light microscopy, MTT, or gel electrophoresis (DNA fragmentation). Investigators may be interested in several products offered by BD Biosciences that may be used for the detection of apoptotic cells by flow cytometry: ApoDirect™ Kit (Cat. No. 556381), ApoBRDU™ Kit (Cat. No. 556405) and Annexin V-FITC (Cat. No. 556420 or 556419). Because these methods vary in sensitivity, it may be necessary to titer the rProtG and/or CD95 NA/LE™ to obtain optimal results.

Notes:
The suggestions given in this procedure are based on conditions that were found to be optimal for the induction of apoptosis by anti-human CD95 (Fas). Studies have shown that the addition of protein G can significantly enhance the efficiency of DX2 in this type of functional assay. It is important to note that there is a great deal of variation between cell lines in the level of apoptosis that can be induced through the Fas receptor. It has been reported that, although some cells express the Fas antigen, they do not necessarily undergo Fas-mediated apoptosis (i.e., the Fas antigen expressed may be anti-Fas sensitive or insensitive). Daudi, Jurkat, and HPB-ALL cells are good positive controls as they are strongly induced by anti-Fas to undergo apoptosis.

Suggested Companion Products

Catalog Number	Name	Size	Clone
555988	FITC Goat Anti-Mouse IgG/IgM	0.5 mg	Polyclonal
554721	Purified NA/LE Mouse IgG1 κ Isotype Control	0.5 mg	107.3
556381	APO-DIRECT™ Kit	50 Tests	(none)
556405	APO-BRDU™ Kit	60 Tests	(none)
556420	FITC Annexin V	100 Tests	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
555899	Lysing Buffer	100 mL	(none)
349202	BD FACS™ Lysing Solution	100 mL	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Cifone MG, De Maria R, Roncaioli P, et al. Apoptotic signaling through CD95 (Fas/Apo-1) activates an acidic sphingomyelinase. *J Exp Med.* 1994; 180(4):1547-1552. (Biology)

Itoh N, Yonehara S, Ishii A, et al. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell.* 1991; 66(2):233-243. (Biology)

Kishimoto T. Tadamitsu Kishimoto .. et al., ed. *Leucocyte typing VI : white cell differentiation antigens : proceedings of the sixth international workshop and conference held in Kobe, Japan, 10-14 November 1996.* New York: Garland Pub.; 1997(Clone-specific)

Schlossman SF. Stuart F. Schlossman .. et al., ed. *Leucocyte typing V : white cell differentiation antigens : proceedings of the fifth international workshop and conference held in Boston, USA, 3-7 November, 1993.* Oxford: Oxford University Press; 1995(Biology)