

Technical Data Sheet

BB700 Mouse Anti-Human CD95

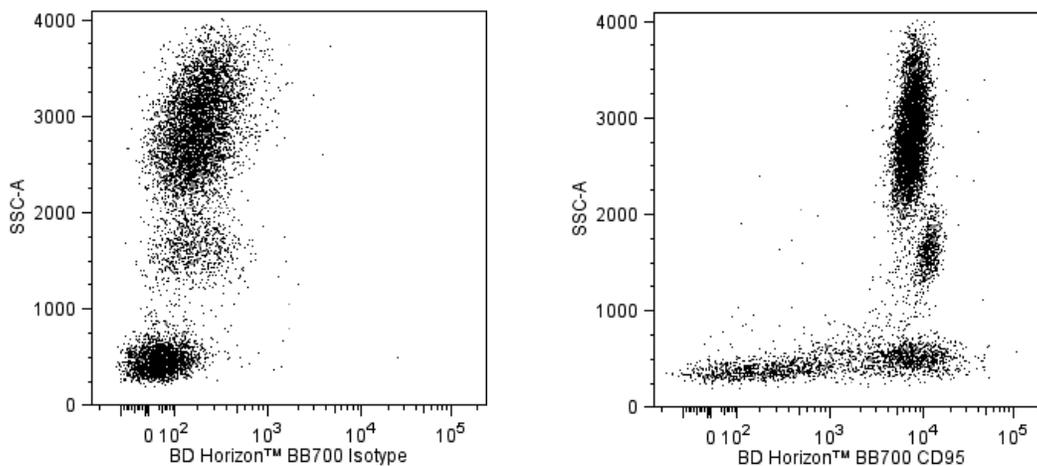
Product Information

Material Number:	566543
Alternate Name:	APO-1; FAS; TNFRSF6; APT1; ALPS1A; FAS1; FASTM; FASLG receptor
Size:	25 Tests
Vol. per Test:	5 µl
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:	Aqueous buffered solution containing ≤0.09% sodium azide.

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.

The antibody was conjugated to BD Horizon BB700, which is part of the BD Horizon Brilliant™ Blue family of dyes. It is a polymer-based tandem dye developed exclusively by BD Biosciences. With an excitation max of 485 nm and an emission max of 693 nm, BD Horizon BB700 can be excited by the 488 nm laser and detected in a standard PerCP-Cy™5.5 set (eg, 695/40-nm filter). This dye provides a much brighter alternative to PerCP-Cy5.5 with less cross laser excitation off the 405 nm and 355 nm lasers.



Multiparameter flow cytometric analysis of CD95 expression on human peripheral blood leucocyte populations. Human whole blood was stained with either BD Horizon™ BB700 Mouse IgG1, κ Isotype Control (Cat. No. 566404; Left Plot) or BD Horizon BB700 Mouse Anti-Human CD95 antibody (Cat. No. 566542/566543; Right Plot). A two-parameter flow cytometric dot plot showing the correlated expression of CD95 (or Ig Isotype control staining) versus side-light scatter (SSC-A) signals was derived from gated events with the forward and side-light scatter characteristics of intact leucocyte populations. Flow cytometric analysis was performed using a BD LSRFortessa™ Cell Analyzer System.

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

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

The antibody was conjugated with BD Horizon™ BB700 under optimum conditions, and unconjugated antibody and free BD Horizon BB700 were removed.

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566543 Rev. 1



Application Notes

Application

Flow cytometry

Routinely Tested

Recommended Assay Procedure:

For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes are used in the same experiment. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. The BD Horizon Brilliant Stain Buffer was designed to minimize these interactions. More information can be found in the Technical Data Sheet for the BD Horizon Brilliant Stain Buffer (Cat. No. 563794/566349) or the BD Horizon Brilliant Stain Buffer Plus (Cat. No. 566385).

When setting up compensation, it is recommended to compare spillover values obtained from cells and BD™ CompBeads to ensure that beads will provide sufficiently accurate spillover values.

For optimal results, it is recommended to perform two washes after staining with antibodies. Cells may be prepared, stained with antibodies and washed twice with wash buffer per established protocols for immunofluorescent staining, prior to acquisition on a flow cytometer. Performing fewer than the recommended wash steps may lead to increased spread of the negative population.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
563794	Brilliant Stain Buffer	100 Tests	(none)
566349	Brilliant Stain Buffer	1000 Tests	(none)
566385	Brilliant Stain Buffer Plus	1000 Tests	(none)
555899	Lysing Buffer	100 mL	(none)
349202	BD FACST™ Lysing Solution	100 mL	(none)
566542	BB700 Mouse Anti-Human CD95	100 Tests	DX2
566404	BB700 Mouse IgG1, κ Isotype Control	50 µg	X40

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1×10^6 cells in a 100-µl experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. The manufacture, use, sale, offer for sale, or import of this product is subject to one or more patents or pending applications. This product, and only in the amount purchased by buyer, may be used solely for buyer's own internal research, in a manner consistent with the accompanying product literature. No other right to use, sell or otherwise transfer (a) this product, or (b) its components is hereby granted expressly, by implication or by estoppel. Diagnostic uses require a separate license.
6. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.
7. BD Horizon Brilliant Blue 700 is covered by one or more of the following US patents: 8,455,613 and 8,575,303.
8. Cy is a trademark of GE Healthcare.
9. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
10. Please refer to www.bdbiosciences.com/pharming/protocols for technical protocols.

References

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- Robertson MJ, Ritz R. Cluster report: CD95. In: 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; 1995:1142-1143. (Biology)
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- Lanier LL, Chang C, Phillips JH. Human NKR-P1A. A disulfide-linked homodimer of the C-type lectin superfamily expressed by a subset of NK and T lymphocytes. *J Immunol*. 1994; 153(6):2417-2428. (Clone-specific: Flow cytometry)