

Technical Data Sheet

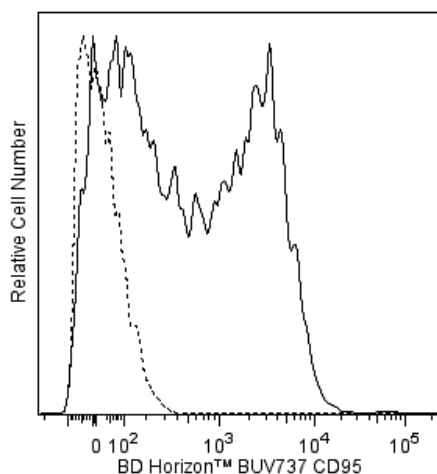
BUV737 Mouse Anti-Human CD95**Product Information**

Material Number:	564710
Alternate Name:	APO-1; FAS; TNFRSF6; APT1; ALPS1A; FAS1; FASTM; FASLG receptor
Size:	100 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 BUV737 which is part of the BD Horizon Brilliant™ Ultraviolet family of dyes. This dye is a tandem fluorochrome with an Ex Max near 350 nm and an Em Max near 737 nm. BD Horizon Brilliant BUV737 can be excited by the ultraviolet laser (355 nm) and detected with a 740/35 nm filter. Due to the excitation of the acceptor dye by the red laser line, there may be significant spillover into red laser detectors with filters in the 700-720 nm range.



Flow cytometric analysis of CD95 expression on human peripheral blood lymphocytes. Whole blood was stained with either BD Horizon™ BUV737 Mouse IgG1, κ Isotype Control (Cat. No. 564299; dashed line histogram) or BD Horizon BUV737 Mouse Anti-Human CD95 antibody (Cat. No. 564710; solid line histogram). Erythrocytes were lysed with BD FACS Lysing Solution (Cat. No. 349202). The fluorescence histogram showing CD95 expression (or Ig Isotype control staining) was derived from events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer 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 BUV737 under optimum conditions, and unconjugated antibody and free BD Horizon BUV737 were removed.

Application Notes**Application**

Flow cytometry

Routinely Tested

BD Biosciences

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564710 Rev. 2



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 of the BD Horizon Brilliant Stain Buffer (Cat. No. 563794/566349) or the BD Horizon Brilliant Stain Buffer Plus (Cat. No. 566385).

BD™ CompBeads can be used as surrogates to assess fluorescence spillover (Compensation). When fluorochrome conjugated antibodies are bound to BD CompBeads, they have spectral properties very similar to cells. However, for some fluorochromes there can be small differences in spectral emissions compared to cells, resulting in spillover values that differ when compared to biological controls. It is strongly recommended that when using a reagent for the first time, users compare the spillover on cells and BD CompBead to ensure that BD CompBeads are appropriate for your specific cellular application.

Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
564299	BUV737 Mouse IgG1, κ Isotype Control	50 μ g	X40
349202	BD FACS™ Lysing Solution	100 mL	(none)
555899	Lysing Buffer	100 mL	(none)
563794	Brilliant Stain Buffer	100 Tests	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
566349	Brilliant Stain Buffer	1000 Tests	(none)
566385	Brilliant Stain Buffer Plus	1000 Tests	(none)

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- μ l 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. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. BD Horizon Brilliant Ultraviolet 737 is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,227,187; 8,575,303; 8,354,239.
7. 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.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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Kishimoto T, Tadimitsu 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: Flow cytometry)

Lanier LL, Chang C, Phillips JH. Human NKRP1A. 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)