Technical Data Sheet

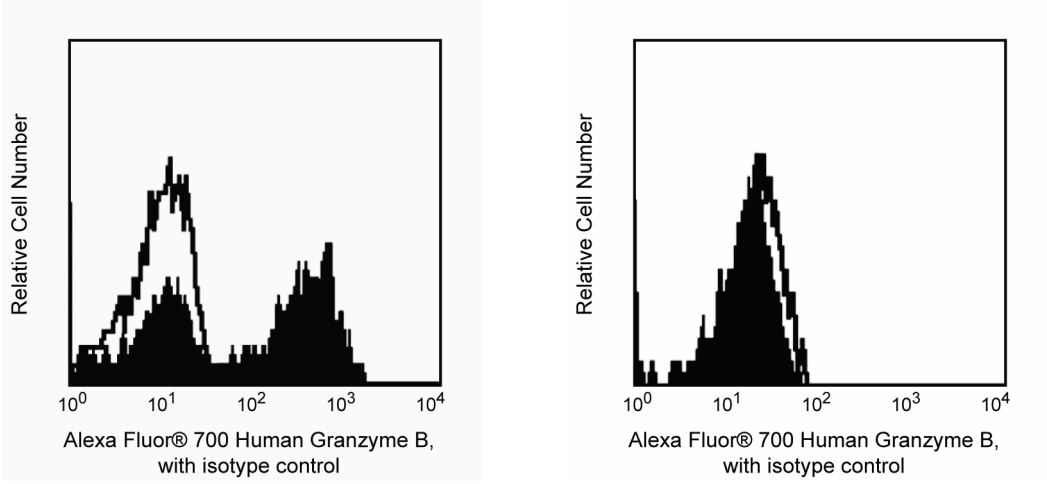
Alexa Fluor® 700 Mouse anti-Human Granzyme B

Product Information

Material Number:	**560213**
Alternate Name:	GZMB; Granzyme-2; CCPI; CGL1; CSPB; CTLA1; CTSGL1; GRB; HLP; SECT
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	GB11
Immunogen:	Human Granzyme B
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium azide.

Description

The GB11 antibody specifically reacts with human granzyme B, a serine protease of approximately 32 kDa. Granzyme B is stored in the granules of cytotoxic T lymphocytes and NK cells along with the pore-forming protein perforin. In the classic model of target cell lysis, perforins create holes in the target cell membrane allowing entrance of granzymes. Granzyme B has been shown to act on specific substrates including caspase-3, -7, -9, and -10 which in turn give rise to enzymes that mediate apoptosis. Granzyme B may also be involved in the hydrolysis of extracellular matrix components. Detectable levels of granzyme B have been detected in sera from healthy volunteers. The immunogen used to generate the GB11 hybridoma was human granzyme B isolated from an NK cell line.



Expression of granzyme B by peripheral blood CD8+ lymphocytes. Whole human blood was lysed with BD Pharm Lyse™ Lysing Buffer (Cat No. 555899) prior to staining with GB11. Whole lysed human blood was subsequently fixed, permeabilized and stained with mouse anti-human granzyme B antibody (Alexa Fluor® 700 GB11, Cat. No. 560213), gated on a positive CD8+ lyomphocytes population (left panel) or gated on negative monocytes cell population (right panel). The open histogram indicates the immunoglobulin isotype control (Alexa Fluor® 700 MOPC-21) used. To demonstrate the specificity of this staining, the binding of Alexa Fluor® 700 GB11 was blocked by preincubation of the fixed/permeabilized cells with excess of an unlabelled GB11 antibody (10 µg, data not shown) prior to stainining. The dot blots were derived from gating on cells with the forward and side scatter characteristics of lymphocytes.

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 to Alexa Fluor® 700 under optimum conditions, and unreacted Alexa Fluor® 700 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

BD Biosciences

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Suggested Companion Products

Catalog Number	Name	Size	Clone
557882	Alexa Fluor® 700 Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
555899	Lysing Buffer	100 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
554722	Fixation and Permeabilization Solution	125 ml	(none)
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 tests	(none)
554723	Perm/Wash Buffer	100 ml	(none)

Product Notices

1. Alexa Fluor® 700 has an adsorption maximum of ~700nm and a peak fluorescence emission of ~720nm. Before staining cells with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
2. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
3. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
4. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
5. 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.
6. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
7. An isotype control should be used at the same concentration as the antibody of interest.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Ronday HK, van der Laan WH, Tak PP et al. Human granzyme B mediates cartilage proteoglycan degradation and is expressed at the invasive front of the synovium in rheumatoid arthritis. *Rheumatology (Oxford)*. 2001; 40:55-61. (Biology)

Smyth MJ, Kelly JM, Sutton VR et al. Unlocking the secrets of cytotoxic granule proteins. *J Leukoc Biol.* 2001; 70:18-29. (Biology)

Spaeny-Dekking EH, Hanna WL, Wolbink AM et al. Extracellular granzymes A and B in humans: detection of native species during CTL responses in vitro and in vivo. *J Immunol.* 1998; 160:3610. (Biology)

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