Technical Data Sheet

PE Mouse Anti-Human Granzyme A Set

Product Information

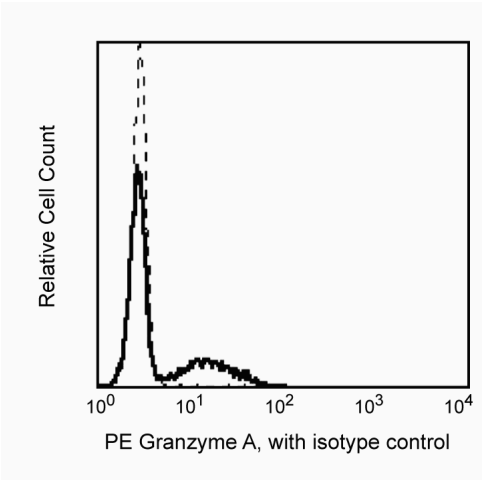
Material Number:	**558904**
Reactivity:	QC Testing: Human
Component:	**51-68395X**
Description:	PE Granzyme A
Size:	100 tests (1 ea)
Vol. per Test:	20 µl
Clone Name:	CB9
Isotype:	Mouse IgG1, κ
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.
Component:	**51-13855X-6**
Description:	PE Mouse IgG1, κ Isotype Control
Size:	100 tests (1 ea)
Vol. per Test:	20 µl
Clone Name:	MOPC-21
Isotype:	Mouse IgG1, κ
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The primary mechanism by which cytotoxic T cells eliminate virally infected cells is by granule exocytosis. The release of cytotoxic granule contents by cytotoxic T lymphocytes (CTL) triggers apoptotic target cell death. CTL granules contain a pore-forming protein, perforin, and a group of serine proteases called granzymes. In the classic model, perforins create holes in the target cell membrane, allowing entrance of the granzymes. Granzyme A and B are the predominant granzymes activated after CTL activation, but each act via an independent apoptotic pathway; granzyme B is activated immediately, while granzyme A acts hours later. Granzyme B does not induce cleavage of caspase-3, lamin B, rho-GTPase or PARP, but does cleave DNA-PKcs and nuclear mitotic apparatus protein (NuMA). Studies involving mice which are deficient in both granzyme A and B suggest a model whereby the granzyme B pathway may have evolved as the major apoptotic pathway with the granzyme A pathway acting as a backup. However, further research is needed to delineate the components of the distinct pathways.

Clone CB9 recognizes human granzyme A. Purified human granzyme A was used as immunogen. Clone MOPC-21 is a mouse IgG1 isotype (negative) control. The MOPC-21 antibody has unknown specificity.

The antibodies are routinely tested in parallel by flow cytometry. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



Profile of permeabilized peripheral blood lymphocytes analyzed by flow cytometry (BDIS, San Jose, CA). *Cells were collected, fixed, and permeabilzied using the Cytofix/Cytoperm™ Kit (554714) for 20 minutes at room temperature (RT), pelleted, and washed twice with Perm/Wash Buffer™ (component of Cat. No. 554714). Cells were resuspended in Perm/Wash Buffer™ and stained with PE Anti-human Granzyme A (Clone CB9) or with an isotype control (clone MOPC-21) for 20-30 minutes at RT in the dark. Cells were then washed once in Perm/Wash Buffer™, resuspended in wash buffer, and analyzed by flow cytometry.*

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Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed by gel filtration chromatography.
Store undiluted at 4° C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554714	BD Cytofix/Cytoperm Fixation/Permeablization Kit	250 tests	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 X 10e6 cells in a 100-µl experimental sample (a test).
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/pharmingen/colors.
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Andrade F, Roy S, Nicholson D, Thornberry N, Rosen A, Casciola-Rosen L. Granzyme B directly and efficiently cleaves several downstream caspase substrates: implications for CTL-induced apoptosis. *Immunity.* 1998; 8(4):451-460.(Biology)

Beresford PJ, Kam CM, Powers JC, Lieberman J. Recombinant human granzyme A binds to two putative HLA-associated proteins and cleaves one of them. *Proc Natl Acad Sci U S A.* 1997; 94(17):9285-9290.(Immunogen: Immunoprecipitation)

Beresford PJ, Xia Z, Greenberg AH, Lieberman J. Granzyme A loading induces rapid cytolysis and a novel form of DNA damage independently of caspase activation. *Immunity.* 1999; 10(5):585-594.(Clone-specific)

Shresta S, Graubert TA, Thomas DA, Raptis SZ, Ley TJ. Granzyme A initiates an alternative pathway for granule-mediated apoptosis. *Immunity.* 1997; 10(5):595-605.(Biology)

Trimble LA, Lieberman J. Circulating CD8 T lymphocytes in human immunodeficiency virus-infected individuals have impaired function and downmodulate CD3 zeta, the signaling chain of the T-cell receptor complex. *Blood.* 1998; 91(2):585-594.(Clone-specific: Flow cytometry)