Technical Data Sheet

PE-CF594 Mouse Anti-Human Perforin

Product Information

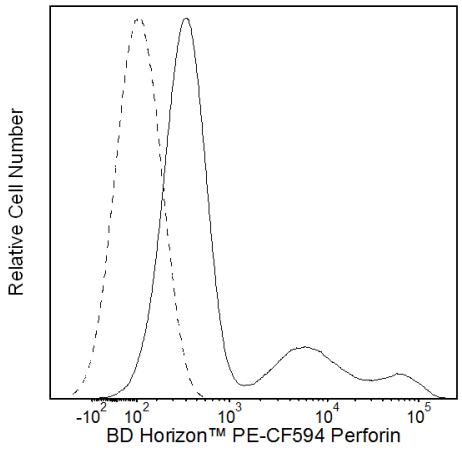
Material Number:	**563763**
Alternate Name:	PRF1; P1; PERF; PFN1; PFP; Perforin-1; Cytolysin; FLH2; HPLH2
Size:	50 tests
Vol. per Test:	5 µl
Clone:	δG9
Immunogen:	Purified Granules from the Human Lymphoma Cell Line YT
Isotype:	Mouse (BALB/c) IgG2b, κ
Reactivity:	QC Testing: Human Reported Reactivity: Bovine
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Perforin has a key role in cell-mediated cytotoxicity. It is a 70 kDa cytolytic protein that is expressed in the cytoplasmic granules of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells. CTLs are involved in eliminating virally infected cells, in anti-tumor immune responses, in allograft rejections, and in some autoimmune diseases. NK cells are important for tumor surveillance and destruction and are involved in allograft rejections. Cytotoxic cells release the contents of their cytotoxic granules, including perforin upon recognition of their target cell. In the presence of calcium, perforin forms transmembrane channels or pores in the membrane of the target cell leading to a cell death that resembles apoptosis. The ability to detect perforin-positive cells with specific antibody should be useful in identifying and understanding perforin-mediated reactions.

Clone δG9 reacts with human and bovine perforin. It does not cross-react with mouse perforin. Purified granules from the human lymphoma cell line YT were used as immunogen. Clone δG9 was initially characterized by immunoprecipitation and immunohistochemistry of frozen tissue sections. The antibody stains scattered lymphocytes in red pulp of spleen, and scattered infiltrated lymphocytes in lymphoma.

This antibody is conjugated to BD Horizon™ PE-CF594, which has been developed exclusively by BD Biosciences as a better alternative to PE-Texas Red®. PE-CF594 excites and emits at similar wavelengths to PE-Texas Red® yet exhibits improved brightness and spectral characteristics. Due to PE having maximal absorption peaks at 496 nm and 564 nm, PE-CF594 can be excited by the blue (488-nm), green (532-nm) and yellow-green (561-nm) lasers and can be detected with the same filter set as PE-Texas Red® (eg 610/20-nm filter).



Flow cytometric analysis of perforin expression in human peripheral blood mononuclear cells. *Human peripheral blood mononuclear cells were fixed and permeabilized with BD Cytofix/Cytoperm™ Fixation and Permeabilization Solution (Cat. No. 554722). The cells were then washed with and stained in BD Perm/Wash™ Buffer (Cat. No. 554723) with either BD Horizon™ PE-CF594 Mouse IgG2b, κ Isotype Control (Cat. No. 562305; dashed line histogram) or BD Horizon™ PE-CF594 Mouse Anti-Human Perforin antibody (Cat. No. 563763; solid line histogram). The fluorescence histograms were derived from gated 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™ PE-CF594 under optimum conditions, and unconjugated antibody and free PE-CF594 were removed.

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
562305	PE-CF594 Mouse IgG2b, κ Isotype Control	0.1 mg	27-35
554722	Fixation and Permeabilization Solution	125 ml	(none)
554723	Perm/Wash Buffer	100 ml	(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-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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
6. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using multi-laser cytometers, which may directly excite both PE and CF™594.
7. When excited by the yellow-green (561-nm) laser, the fluorescence may be brighter than when excited by the blue (488-nm) laser.
8. Texas Red is a registered trademark of Molecular Probes, Inc., Eugene, OR.
9. CF™ is a trademark of Biotium, Inc.
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11. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
12. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Endsley JJ, Furrer JL, Endsley MA, McIntosh MA, Maue AC, Waters WR, et al. Characterization of bovine homologues of granulysin and NK-lysin. *J Immunol.* 2004; 173(4):2607-2614. (Clone-specific: Flow cytometry, Western blot)

Fox WM 3rd, Hameed A, Hutchins GM, et al. Perforin expression localizing cytotoxic lymphocytes in the intimas of coronary arteries with transplant-related accelerated arteriosclerosis. *Hum Pathol.* 1993; 24(5):477-482. (Clone-specific: Immunohistochemistry)

Hameed A, Fox WM, Kurman RJ, Hruban RH, Podack ER. Perforin expression in endometrium during the menstrual cycle. *Int J Gynecol Pathol.* 1995; 14(2):143-150. (Clone-specific: Flow cytometry)

Hameed A, Fox WM, Kurman RJ, Hruban RH, Podack ER. Perforin expression in human cell-mediated luteolysis. *Int J Gynecol Pathol.* 1995; 14(2):151-157. (Clone-specific: Immunohistochemistry)

Hameed A, Olsen KJ, Cheng L, Fox WM 3rd, Hruban RH, Podack ER. Immunohistochemical identification of cytotoxic lymphocytes using human perforin monoclonal antibody. *Am J Pathol.* 1992; 140(5):1025-1030. (Immunogen: Immunohistochemistry, Immunoprecipitation)

Hameed A, Podack ER, Fox WM, Schafer RW, Sherman ME. Detection of perforin in human peritoneal fluid T-lymphocytes. *Acta Cytol.* 1996; 40(3):401-407. (Clone-specific: Immunohistochemistry)

Rukavina D, Balen-Marunic S, Rubesa G, Orlic P, Vujaklija K, Podack ER. Perforin expression in peripheral blood lymphocytes in rejecting and tolerant kidney transplant recipients. *Transplantation.* 1996; 61(2):285-291. (Clone-specific: Flow cytometry)

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