Technical Data Sheet

PE-Cy™5 Mouse Anti-Human CD152

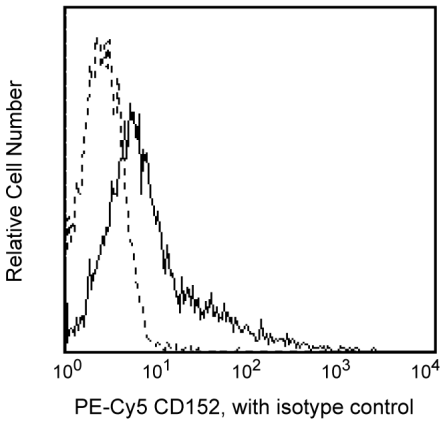
Product Information

Material Number:	**561717**
Alternate Name:	CTLA-4
Size:	25 tests
Vol. per Test:	20 µl
Clone:	BNI3
Isotype:	Mouse IgG2a, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Reacts with the "cytolytic T lymphocyte-associated antigen", CTLA-4. CTLA-4 is transiently expressed on activated CD28+ T cells and binds to CD80 and CD86 present on antigen presenting cells (APC) with high avidity. This interaction appears to deliver a negative regulatory signal to the T cell. There are recent reports that indicate that CTLA-4 is also expressed on B cells when cultured with activated T cells, suggesting a possible role of CTLA-4 in the regulation of B-cell response. Immobilized BNI3.1 enhances T-cell proliferation induced by CD3 and CD28.

Recent studies have showed that CD152 can be expressed by regulatory T (Treg) cells. It has been found this antibody can stain the intracellular CD152 on the Treg cells after fixation and permeabilization of cells.



Profile of concanavalin-A-stimulated peripheral blood mononuclear cells analyzed on a BD FACScan™ (BDIS, San Jose, CA)

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 PE-Cy5 (formerly known as BD Cy-Chrome™) under optimum conditions, and unconjugated antibody and free PE-Cy5 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested
Intracellular staining (flow cytometry)	Tested During Development

Suggested Companion Products

Catalog Number	Name	Size	Clone
555575	PE-Cy™5 Mouse IgG2a, κ Isotype Control	100 tests	G155-178
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 tests	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

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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. 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/colors.
5. PE-Cy5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the PE-Cy5 tandem fluorochrome, extra care must be taken when using dual-laser cytometers which may directly excite both PE and Cy5™.
6. PE-Cy5 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by the 488 nm light of an Argon ion laser and serves as an energy donor, coupled to the cyanine dye Cy5, which acts as an energy acceptor and fluoresces at 670 nm. BD Biosciences Pharmingen has maximized the fluorochrome energy transfer in PE-Cy5, thus maximizing its fluorescence emission intensity, minimizing residual emission from PE, and minimizing lot-to-lot variation.
7. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
8. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
9. 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.
10. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
11. 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.
12. PE-Cy5 tandem fluorochromes have been reported to bind some classes of human macrophages and granulocytes via Fc receptors, and PE has been reported to bind to mouse B lymphocytes via Fc receptors. Preincubation of mouse leukocytes with Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 can reduce the non-specific binding of PE-Cy5-conjugated reagents to mouse B cells. However, PE-Cy5 conjugated reagents should not be used to stain splenocytes of SJL, NOD, and MRL mice as B lymphocytes and/or other leukocytes have been reported to non-specifically stain regardless of the use of Mouse BD Fc Block™ (the CD72c complex has been implicated for PE-Cy5 binding in these strains). Reagents conjugated to PE, PerCP, PerCP-Cy5.5, APC, and APC-Cy7 tandem fluorochrome can be used on leukocytes from these mouse strains.
13. An isotype control should be used at the same concentration as the antibody of interest.

References

Barclay NA, Brown MH, Birkeland ML, et al, ed. *The Leukocyte Antigen FactsBook.* San Diego, CA: Academic Press; 1997. (Biology)

Kuiper HM, Brouwer M, Linsley PS, van Lier RA. Activated T cells can induce high levels of CTLA-4 expression on B cells. *J Immunol.* 1995; 155(4):1776-1783. (Biology)

Lindsten T, Lee KP, Harris ES, et al. Characterization of CTLA-4 structure and expression on human T cells. *J Immunol.* 1993; 151(7):3489-3499. (Biology)

Morton PA, Fu XT, Stewart JA, et al. Differential effects of CTLA-4 substitutions on the binding of human CD80 (B7-1) and CD86 (B7-2). *J Immunol.* 1996; 156(3):1047-1054. (Biology)