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)](image)

**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**

<table>
<thead>
<tr>
<th>Flow cytometry Routinely Tested</th>
<th>Intracellular staining (flow cytometry) Tested During Development</th>
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**Suggested Companion Products**

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>555575</td>
<td>PE-Cy™5 Mouse IgG2a, κ Isotype Control</td>
<td>100 tests</td>
<td>G155-178</td>
</tr>
<tr>
<td>554714</td>
<td>BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit</td>
<td>250 tests</td>
<td>(none)</td>
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<tr>
<td>554656</td>
<td>Stain Buffer (FBS)</td>
<td>500 ml</td>
<td>(none)</td>
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</table>

**BD Biosciences**

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561717 Rev. 1
Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use \( 1 \times 10^6 \) 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 wwwbdbiosciences.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.

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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


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)