The EH12.1 monoclonal antibody specifically binds to CD279 which is also known as Programmed cell death 1 (PD1). CD279 is an immunoregulatory receptor expressed on activated T cells, B cells, and myeloid cells. It contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) in the cytoplasmic region. Mice deficient in CD279 show a breakdown of peripheral tolerance and manifest multiple autoimmune symptoms. PD-L1 and PD-L2 are ligands of CD279 and members of the B7 gene family. CD279:PD-Ligands interaction inhibits T cell proliferation and cytokine secretion. Reports suggest that the B7/CTLA-4 pathway primarily attenuates, limits, and/or terminates naïve T-cell activation in secondary lymphoid organs. The PD-ligand:CD279 pathway, on the other hand, may primarily attenuate, limit, and/or terminate T-, B-, and myeloid cell activation/effector function at sites of inflammation in the periphery.

The antibody was conjugated to BD Horizon BB700, which is part of the BD Horizon Brilliant™ Blue family of dyes. It is a polymer-based tandem dye developed exclusively by BD Biosciences. With an excitation max of 485 nm and an emission max of 693 nm, BD Horizon BB700 can be excited by the 488 nm laser and detected in a standard PerCP-Cy™5.5 set (e.g., 695/40-nm filter). This dye provides a much brighter alternative to PerCP-Cy5.5 with less cross laser excitation off the 405 nm and 355 nm lasers.

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™ BB700 under optimum conditions, and unconjugated antibody and free BD Horizon BB700 were removed.
Application Notes

Application

Flow cytometry Routinely Tested

Recommended Assay Procedure:
For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes are used in the same experiment. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. The BD Horizon Brilliant Stain Buffer was designed to minimize these interactions. More information can be found in the Technical Data Sheet for the BD Horizon Brilliant Stain Buffer (Cat. No. 563794/566349) or the BD Horizon Brilliant Stain Buffer Plus (Cat. No. 566385).

When setting up compensation, it is recommended to compare spillover values obtained from cells and BD™ CompBeads to ensure that beads will provide sufficiently accurate spillover values.

For optimal results, it is recommended to perform two washes after staining with antibodies. Cells may be prepared, stained with antibodies and washed twice with wash buffer per established protocols for immunofluorescent staining, prior to acquisition on a flow cytometer. Performing fewer than the recommended wash steps may lead to increased spread of the negative population.

Suggested Companion Products

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<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
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<tbody>
<tr>
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<td>Stain Buffer (FBS)</td>
<td>500 mL</td>
<td>(none)</td>
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<tr>
<td>554657</td>
<td>Stain Buffer (BSA)</td>
<td>500 mL</td>
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<td>563794</td>
<td>Brilliant Stain Buffer</td>
<td>100 Tests</td>
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<tr>
<td>566349</td>
<td>Brilliant Stain Buffer</td>
<td>1000 Tests</td>
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<tr>
<td>349202</td>
<td>BD FACSTM Lysing Solution</td>
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<tr>
<td>555899</td>
<td>Lysing Buffer</td>
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<td>566385</td>
<td>Brilliant Stain Buffer Plus</td>
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<tr>
<td>555332</td>
<td>FITC Mouse Anti-Human CD3</td>
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<td>UCHT1</td>
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<td>FITC Mouse Anti-Human CD3</td>
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<td>500 Tests</td>
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<td>566461</td>
<td>BB700 Mouse Anti-Human CD279 (PD-1)</td>
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<td>EH12.1</td>
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<td>566404</td>
<td>BB700 Mouse IgG1, κ Isotype Control</td>
<td>50 µg</td>
<td>X40</td>
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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. 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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. The manufacture, use, sale, offer for sale, or import of this product is subject to one or more patents or pending applications. This product, and only in the amount purchased by buyer, may be used solely for buyer’s own internal research, in a manner consistent with the accompanying product literature. No other right to use, sell or otherwise transfer (a) this product, or (b) its components is hereby granted expressly, by implication or by estoppel. Diagnostic uses require a separate license.
6. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.
7. BD Horizon Brilliant Blue 700 is covered by one or more of the following US patents: 8,455,613 and 8,575,303.
8. Cy is a trademark of GE Healthcare.

References
Bennett F, Luxenberg D, Ling V, et al. Program death-1 engagement upon TCR activation has distinct effects on costimulation and cytokine-driven proliferation: attenuation of ICOS, IL-4, and IL-21, but not CD28, IL-7, and IL-15 responses. J Immunol. 2003; 170(2);711-718. (Biology)