

Technical Data Sheet

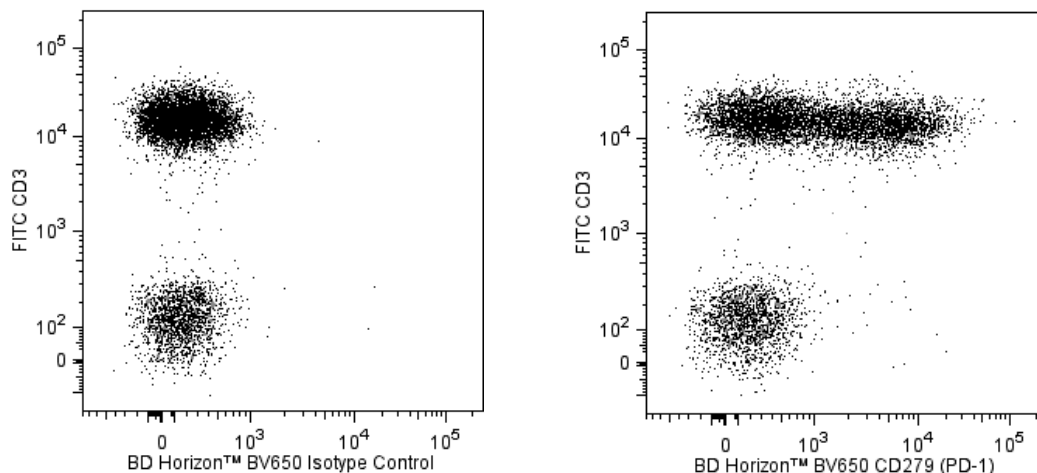
BV650 Mouse Anti-Human CD279 (PD-1)**Product Information**

Material Number:	564104
Alternate Name:	hPD-1; PD1; PDCD1; Programmed cell death 1; SLEB2
Size:	50 tests
Vol. per Test:	5 µl
Clone:	EH12.1 (also known as EH12)
Immunogen:	Human PD-1 Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The EH12.1 monoclonal antibody specifically binds to CD279. CD279 is an immunoregulatory receptor that is expressed on activated T cells, B cells and myeloid cells and 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 are members of the B7 gene family. Interaction of CD279:PD-Ligands results in inhibition of T cell proliferation and cytokine secretion. Reports suggest that the B7/CTLA-4 pathway functions primarily to attenuate, limit, and/or terminate 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™ BV650 which is part of the BD Horizon Brilliant Violet™ family of dyes. This dye is a tandem fluorochrome of BD Horizon BV421 with an Ex Max of 405-nm and an acceptor dye with an Em Max at 650-nm. BD Horizon BV650 can be excited by the violet laser and detected in a filter used to detect APC-like dyes (eg, 660/20-nm filter). Due to the excitation and emission characteristics of the acceptor dye, there will be spillover into the APC and Alexa Fluor® 700 detectors. However, the spillover can be corrected through compensation as with any other dye combination.



Two-color flow cytometric analysis of CD279 expression on human peripheral blood lymphocytes. Human whole blood was stained with FITC Mouse Anti-Human CD3 antibody (Cat. No. 555332/561806/561807) and either BD Horizon™ BV650 Mouse IgG1, κ Isotype Control (Cat. No. 563231; Left Panel) or BD Horizon BV650 Mouse Anti-Human CD279 (PD-1) antibody (Cat. No. 564104; Right Panel). The erythrocytes were lysed with BD FACS™ Lysing Solution (Cat. No. 349202). The two-color flow cytometric dot plots show the correlated expression of CD279 (or Ig Isotype control staining) versus CD3 for gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometric analysis was performed using a BL SRFortessa™ Cell Analyzer System.

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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™ BV650 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV650 were removed.

Application Notes

Application

Flow cytometry

Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
554657	Stain Buffer (BSA)	500 ml	(none)
563231	BV650 Mouse IgG1, k Isotype Control	50 µg	X40
555332	FITC Mouse Anti-Human CD3	100 tests	UCHT1
561806	FITC Mouse Anti-Human CD3	25 tests	UCHT1
561807	FITC Mouse Anti-Human CD3	500 tests	UCHT1
349202	BD FACST™ Lysing Solution	100 ml	(none)
555899	Lysing 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. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
6. Brilliant Violet™ 650 is a trademark of Sirigen.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. Please refer to www.bdbiosciences.com/pharming/en/protocols for technical protocols.

References

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Carter L, Fouser LA, Jussif J, et al. PD-1:PD-L inhibitory pathway affects both CD4(+) and CD8(+) T cells and is overcome by IL-2. *Eur J Immunol.* 2002; 32:634-643. (Biology)

Dorfman DM, Brown JA, Shahsafaei A, Freeman GJ. Programmed death-1 (PD-1) is a marker of germinal center-associated T cells and angioimmunoblastic T-cell lymphoma. *Am J Surg Pathol.* 2006; 30:802-810. (Immunogen: Flow cytometry, Immunohistochemistry)

Freeman GJ, Long AJ, Iwai Y, et al. Engagement of PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med.* 2000; 192:1027-1034. (Biology)

Kanai T, Totsuka T, Uraushihara K, et al. Blockade of B7-H1 suppresses the development of chronic intestinal inflammation. *J Immunol.* 2003; 171(8):4156-4163. (Biology)

Latchman Y, Wood CR, Chernova T, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat Immunol.* 2001; 2(3):261-268. (Biology)

Velu V, Kannanganat S, Ibegbu C, et al. Elevated expression levels of inhibitory receptor programmed death 1 on simian immunodeficiency virus-specific CD8 T cells during chronic infection but not after vaccination. *J Virol.* 2007; 81(11):5819-5828. (Clone-specific: Blocking, Flow cytometry, Functional assay, Inhibition)

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