Technical Data Sheet

APC Rat Anti-Mouse CD274

Product Information

Material Number: 564715
Alternate Name: B7-H1, PD-L1; PD1L1; Programmed death ligand 1
Size: 50 µg
Concentration: 0.2 mg/ml
Clone: MIH5
Immunogen: DBA/2 mouse lymphoma L5178Y transfected with Pdcd1lg1 cDNA
Isotype: Rat (SD) IgG2a, λ
Reactivity: QC Testing: Mouse
Storage Buffer: Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The MIH5 antibody reacts with CD274, also known as B7-H1 or PDL1, a 43-kDa glycoprotein encoded by the Pdcd1lg1 gene of the B7 family of the Ig superfamily. Pdcd1lg1 mRNA is expressed in more tissues than other members of the B7 family; transcripts are found in lymphoid tissues and many, but not all, non-lymphoid tissues. The protein has been detected at low levels on resting peripheral T and B lymphocytes, macrophages, and dendritic cells. B7-H1 mRNA and protein expression are upregulated upon activation of T and B cells, macrophages, dendritic cells, and epidermal keratinocytes by a variety of stimulatory factors. B7-H1’s receptor, PD-1, contains an ITIM (Immunoreceptor Tyrosine-based Inhibitory Motif) on its intracytoplasmic region and is expressed on activated B and T lymphocytes, suggesting that B7-H1-PD-1 interaction may be involved in the negative regulation of immune responses. The second PD-1 ligand, B7-DC (PD-L2), is also a member of the B7 family of the Ig superfamily. Furthermore, B7-H1 may participate in positive immunoregulation, or costimulation of T cells, through an additional receptor, which is not PD-1 and distinct from the alternate receptor for B7-DC. The MIH5 antibody blocks the binding of PD-1-Ig to B7-H1 transfectants.

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 to APC under optimum conditions, and unconjugated antibody and free APC were removed.

Two-color flow cytometric analysis of CD274 expression on resting and activated mouse splenocytes. Mouse splenic leucocytes were either not activated (Upper Panels) or activated (Lower Panels) by culture with plate-bound Purified NA/LE Hamster Anti-Mouse CD3 antibody (Cat. No. 553057) for 3 days (37°C). The cells were preincubated with Purified Rat Anti-Mouse CD16/CD32 antibody (Mouse BD Fc Block™) (Cat. No. 553141/553142). The cells were then stained with FITC Rat Anti-Mouse CD4 antibody (Cat. No. 553729/557307/561828) and either APC Rat IgG2a, λ Isotype Control (Cat. No. 560720; Left Panels) or APC Rat Anti-Mouse CD274 antibody (Cat. No. 564715; Right Panels). Two-color flow cytometric contour plots showing the correlated expression of CD274 (or Ig isotype control staining) versus CD4 were derived from gated events with the forward and side light-scatter characteristics of viable leucocytes. Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.
Application Notes

Application Flow cytometry Routinely Tested

Suggested Companion Products

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<td>Stain Buffer (FBS)</td>
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<td>554657</td>
<td>Stain Buffer (BSA)</td>
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Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
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. This APC-conjugated reagent can be used in any flow cytometer equipped with a dye, HeNe, or red diode laser.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at wwwbdbiosciencescom/colors.
6. Please refer to wwwbdbiosciencescom/pharlingen/protocols for technical protocols.

References


