

Reagents Provided

Allophycocyanin (APC)-conjugated goat polyclonal anti-mouse B7-H1: Supplied as 10 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Isotype: goat IgG

Reagents Not Provided

- Flow Cytometry Staining Buffer (Catalog # FC001) or other BSA-supplemented saline buffer.

Storage

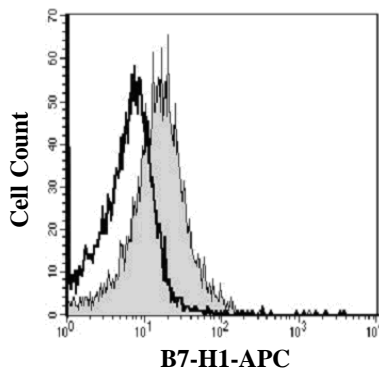
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing B7-H1 within a population and qualitatively determine the density of B7-H1 on cell surfaces by flow cytometry.

Product Description

Produced in goats immunized with purified, NS0-derived, recombinant mouse B7 homolog 1 (rmB7-H1) extracellular domain. Mouse B7-H1 specific IgG was purified by mouse B7-H1 affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Cell surface expression of B7-H1 is determined by flow cytometry using 620 - 650 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.



Mouse splenocytes were stained with APC-conjugated anti-mouse B7-H1 (Catalog # FAB1019A, filled histogram) or isotype control (Catalog # IC108A, open histogram).

Background Information

B7-H1, also known as PD-L1 and CD274, is a type I transmembrane protein belonging to the B7 family. B7-H1 contains one Ig-like C2-type domain and one Ig-like V-type domain. It is constitutively expressed in several tissues and is upregulated on various tumors and activated dendritic cells, macrophages, and B cells. B7-H1 interacts with PD-1 on T cells to limit T cell activation and regulate immune tolerance. Within the extracellular region, mouse and human B7-H1 share 73% amino acid sequence identity.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using mouse splenocytes.

- Cells may be Fc-blocked with 1 µg of mouse IgG/10⁵ cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
- After blocking, 10 µL of conjugated antibody was added to 1 - 2.5 x 10⁵ cells and incubated for 30 minutes at room temperature.
- Unbound antibody was removed by washing the cells twice in Flow Cytometry Staining Buffer (Catalog # FC001). Note that whole blood requires a RBC lysis step at this point using Flow Cytometry Mouse Lyse Buffer (Catalog # FC003).
- The cells were resuspended in Flow Cytometry Staining Buffer for final analysis by flow cytometry. As a control for this analysis, cells in a separate tube should be treated with APC-labeled goat IgG antibody. This procedure may need to be modified, depending upon cell type and final utilization. Individual users may need to titrate to determine optimal reagent amount for their specific use.

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.