Monoclonal Anti-human B7-H3-Allophycocyanin

Catalog Number: FAB1027A
Lot Number: AAPJ01
100 Tests

Reagents Provided

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human B7-H3: Supplied as 10 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 185504

Isotype: mouse IgG_1

Reagents Not Provided

- PBS (Dulbecco's PBS)
- BSA

Storage

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

Intended Use

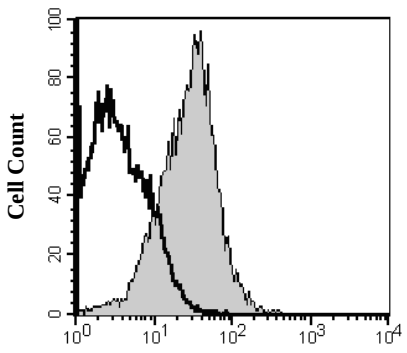
Designed to quantitatively determine the percentage of cells bearing B7-H3 within a population and qualitatively determine the density of B7-H3 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the Allophycocyanin-labeled monoclonal antibody, which binds to cells expressing B7-H3. Unbound allophycocyanin-conjugated antibody is then washed from the cells. Cells expressing B7-H3 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of B7-H3. Cell surface expression of B7-H3 is determined by flow cytometry using 620 - 650 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.

Reagent Preparation

Allophycocyanin-conjugated mouse anti-human B7-H3: Use as is; no preparation necessary.



PC-3 cells were stained with APC-conjugated anti-human B7-H3 (Catalog # FAB1027A, filled histogram) or isotype control (Catalog # IC002A, open histogram).

Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anticoagulant. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) followed by centrifugation at 500 x g for 5 minutes. 50 μL of packed cells should then be transferred to a 5 mL tube for staining with the monoclonal antibody. Whole blood will require lysis of RBC following the staining procedure.

Cell Cultures: Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA) to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4×10^6 cells/mL and 25 μL of cells (1×10^5) transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from their substrates. Cells that require trypsinization to enable removal from their substrates should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

Sample Staining

1) Cells should be Fc-blocked by treatment with 1 μg of human IgG/10^5 cells for 15 minutes at room temperature prior to staining. Do not wash excess blocking IgG from this reaction.
2) Transfer 25 μL of the Fc-blocked cells (1×10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of APC-conjugated B7-H3 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted B7-H3 reagent by washing the cells twice in 4 mL of the same PBS buffer (*note: whole blood will require an RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Whole Blood Lysing Kit, Catalog # WL1000*).
6) Finally, resuspend the cells in 200 - 400 μL of PBS buffer for analysis by flow cytometry.
7) As a control for analysis, cells in a separate tube should be treated with APC-labeled mouse IgG_1 antibody.

This procedure may need modification, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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Background Information

T cells require a signal induced by the engagement of the T cell receptor and a “costimulatory” signal(s) through distinct T cell surface molecules for optimal T cell expansion and activation. Members of the B7 superfamily of counter-receptors were identified by their ability to interact with costimulatory molecules found on the surface of T cells. Members of the B7 superfamily include B7-1 (CD80), B7-2 (CD86), B7-H1 (PD-L1), B7-H2 (B7RP-1), PD-L2 (B7-DC), and B7-H3.[1] Human B7-H3 was originally identified as a 316 amino acid (aa) type I membrane precursor protein with a putative 28 aa signal peptide, a 217 aa extracellular region containing one V-like and one C-like Ig domain, a transmembrane region, and a 45 aa cytoplasmic domain.[2] Subsequently, a second dominantly expressed form of human B7-H3 containing a splice variation that duplicates the V-like and C-like Ig domain was found. The 534 aa splice variant of B7-H3 has been referred to as B7-H3b, 4Ig-B7-H3, and B7-H3VCVC.[3 - 5] RT-PCR transcripts for both B7-H3 and 4-IG-B7-H3 have been found in all tissues except peripheral blood leukocytes.[5] Southern blot analysis indicates that the 4-IG-B7-H3 isoform of B7-H3 is the predominate isoform expressed in human tissues.[5] In mouse, only a single form of B7-H3 containing one V-like and one C-like Ig domain was detected.[5] Mouse and human B7-H3 share 87% aa sequence identity.[3] B7-H3 has been shown to be expressed at very high levels in immature dendritic cells, at moderate levels on mature dendritic cells, LPS-stimulated immature dendritic cells, and LPS-stimulated monocytes, and at low levels on resting monocytes.[3] B7-H3 binds to activated T cells via an as yet unidentified receptor. In assays using sub-optimal amounts of anti-CD3 stimulation, 2-Ig-B7-H3 enhances T cell proliferation, T cell interferon-γ (IFN-γ) production, and induction of cytotoxic T cells.[2] In an assay in which cells were transfected with 4-Ig-B7-H3 or 2-Ig-B7-H3 and anti-CD3 antibody was added, neither 4Ig-B7-H3 nor 2Ig-B7-H3 was capable of co-stimulating T cell proliferation or IFN-γ production.[4, 5]

References

1. Coyle, A.J. and J.-C. Gutierrez-Ramos, 2001, Nature Immunol. **2**:203 - 209.
2. Chapoval, A.I. *et al.*, 2001, Nature Immunol. **2**:269 - 274.
3. Sun, M. *et al.*, 2002. J. Immunol. **168**:6294 - 6297.
4. Steinberger, *et al.*, 2004. J. Immunol. **172**:2352 - 2359.
5. Ling, V. *et al.*, 2003, Genomic **82**: 365 - 377.

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.