

## Reagents Provided

### Allophycocyanin (APC)-conjugated mouse monoclonal anti-human

**CD53:** Supplied as 10 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

**Clone #:** 425514

**Isotype:** mouse IgG<sub>2A</sub>

## Reagents Not Provided

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

## Storage

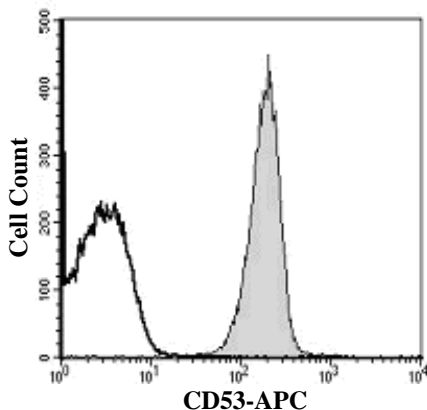
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

## Intended Use

Designed to quantitatively determine the percentage of cells bearing CD53 within a population and qualitatively determine the density of CD53 on cell surfaces by flow cytometry.

## Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with NS0 cells transfected with human CD53 (Accession # P19397). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Cell surface expression of CD53 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.



PBMC granulocytes were stained with APC-conjugated anti-human CD53 (Catalog # FAB4624A, filled histogram) or isotype control (Catalog #

IC008A, open histogram).

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2009/09/25

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

## Background Information

CD53, also known as TSPAN25, is a 35 kDa - 40 kDa cell surface and endosomal membrane glycoprotein in the tetraspanin superfamily. CD53 is widely expressed on hematopoietic cells. Ligation of CD53 promotes cell activation and survival as well as homotypic cell-cell adhesion. CD53 associates with other tetraspanins, MHC class I and II molecules, and integrin  $\alpha 4\beta 1$ . Human CD53 shares 81% - 83% amino acid sequence identity with mouse and rat CD53.

## Flow Cytometry Validation

This antibody has been tested for flow cytometry using PBMC granulocytes.

- Cells may be Fc-blocked with 1 µg of human IgG/10<sup>5</sup> 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 10<sup>5</sup> 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 Human Lyse Buffer (Catalog # FC002).
- The cells were resuspended in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with APC-labeled mouse IgG<sub>2A</sub> antibody. This procedure may need to be modified, depending upon cell type and final utilization. Individual users may need to titrate to determine the 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.