

## Reagents Provided

**Phycoerythrin (PE)-conjugated mouse monoclonal anti-human BST2/Tetherin:** Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

**Clone #:** 696739

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

## Reagents Not Provided

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

## Storage

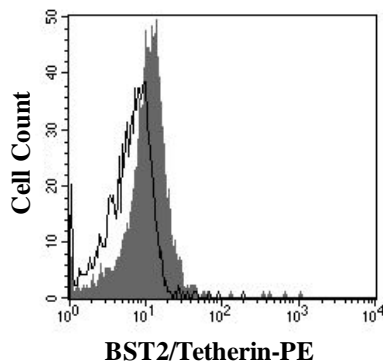
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

## Intended Use

Designed to quantitatively determine the percentage of cells bearing BST2/Tetherin within a population and qualitatively determine the density of BST2/Tetherin 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 human BST2/Tetherin synthetic peptide (Accession # Q10589). The IgG fraction of the tissue culture supernatant was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to PE fluorochrome. Cell surface expression of BST2/Tetherin is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 565-605 nm.



Human peripheral blood monocytes were stained with PE-conjugated anti-human BST2/Tetherin (Catalog # FAB7609P; filled histogram) or PE-conjugated isotype control (Catalog # IC003P; open histogram).

## Background Information

BST2, also known as Tetherin or PDCA1 and designated CD317, is a 30-35 kDa interferon-inducible protein that associates with the plasma membrane with its transmembrane segment and GPI anchor. BST2 is expressed on bone marrow stromal cells and is upregulated in breast cancer and astrocytoma. It binds to ILT7 on plasmacytoid dendritic cells and inhibits pro-inflammatory TLR7 and TLR9 signaling. BST2 inhibits the release of Kaposi sarcoma virus, HIV-1, and Lassa virus from infected cells, but this function is counteracted by viral proteins, which directly bind and trigger the degradation of BST2. Human BST2 is synthesized with a 20 aa cytoplasmic domain, a 28 aa transmembrane segment, a 113 aa extracellular domain, and a 19 aa C-terminal propeptide. Following removal of the propeptide BST2 is modified with a GPI anchor at Ser161. Within the peptide immunogen which represents aa 103-117 of the ECD, human BST2 shares 28% aa sequence identity with mouse and rat BST2.

## Flow Cytometry Validation

This antibody has been tested for flow cytometry using human peripheral blood monocytes.

- 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 up to 1 x 10<sup>6</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 PE-labeled mouse IgG<sub>2A</sub> antibody. This procedure may need to be modified, depending upon the 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.