

## Reagents Provided

**Allophycocyanin-conjugated rat monoclonal anti-mouse BAFF:**  
Supplied as 10 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

**Clone #:** 121808

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

## Reagents Not Provided

**Flow Cytometry Fixation Buffer** (Catalog # FC004) or other 4% paraformaldehyde fixation buffer.

**Flow Cytometry Permeabilization/Wash Buffer I (1X)** (Catalog # FC005) or other saponin-containing saline buffer.

## Storage

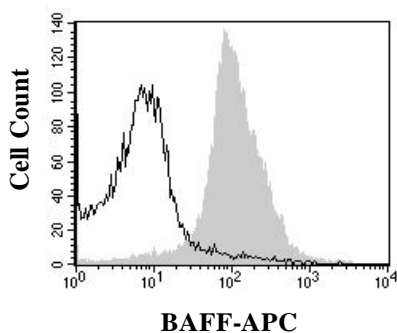
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

## Intended Use

Designed to quantitatively determine the percentage of cells containing BAFF within a population and qualitatively determine the density of intracellular BAFF 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 rat immunized with purified NS0-derived recombinant mouse BAFF (rmBAFF; aa 127-309; Accession # Q9WU72). The IgG fraction of tissue culture supernatant was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Intracellular expression of BAFF is determined by flow cytometry using 620-650 nm wavelength excitation range and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660-670 nm.



Mouse splenocytes cells were stained with APC-conjugated anti-mouse BAFF (Catalog # IC1357A; filled histogram) or APC-conjugated isotype control (Catalog # IC006A; open histogram).

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

## Background Information

BAFF (also known as TALL1, BLyS, and THANK) is a type II transmembrane glycoprotein belonging to the TNF superfamily and has been designated TNF superfamily member 13B (TNFSF13B). Mouse BAFF is a 309 amino acid (aa) protein consisting of a 248 aa extracellular domain, a 21 aa transmembrane region and a 45 aa cytoplasmic tail.<sup>1,2</sup> BAFF has the typical structural characteristics of TNF superfamily ligands. It is a homotrimeric protein having the structurally conserved motif known as TNF homology domain.<sup>3,4</sup> BAFF is expressed in peripheral blood mononuclear cells, in spleen and lymph nodes. Its expression in resting monocytes is up-regulated by IFN- $\alpha$ , IFN- $\beta$ , LPS, and IL-10. BAFF provides critical survival signals to a subset of B cells with intermediate maturation status (T2 B cells) during the immune response.<sup>5</sup>

## References

- Schneider, P. *et al.* (1999) *J. Exp. Med.* **189**:1747.
- Mukhopadhyay, A. *et al.* (1999) *J. Biol. Chem.* **274**:15978.
- Karpusas, M. *et al.* (2002) *J. Mol. Biol.* **315**:1145.
- Liu, Y. *et al.* (2002) *Cell* **108**:383.
- Batten, M. *et al.* (2000) *J. Exp. Med.* **192**:1453.

## Flow Cytometry Validation

For intracellular staining, cells must first be fixed and permeabilized. We recommend the use of 4% PFA as a fixative and a 0.1% saponin balanced salt solution for permeabilization and washing (see Reagents Not Provided).

- Cells were harvested and washed twice in saline buffer.
- Cell surface staining may be done at this point following the manufacturer's staining procedure.
- Up to  $1 \times 10^6$  cells were resuspended in 0.5 mL of cold Flow Cytometry Fixation Buffer (Catalog # FC004) and incubated at room temperature for 10 minutes.
- Following fixation, the cells were washed twice in saline buffer, then once in Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005).
- After permeabilization, 10 µL of conjugated antibody was added and the cells were incubated for 30 minutes at room temperature **in the dark**.
- The cells were washed twice with Flow Cytometry Permeabilization/Wash Buffer I.
- The cells were resuspended in saline buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with APC-labeled rat IgG<sub>2A</sub> antibody. This procedure may need to be modified, depending on 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.