

## Reagents Provided

**Alexa Fluor® 488-conjugated rat monoclonal anti-mouse Galectin-3:**  
Supplied as 10 µg of antibody in 0.5 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

**Clone #:** 202213

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

## Reagents Not Provided

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

## Storage

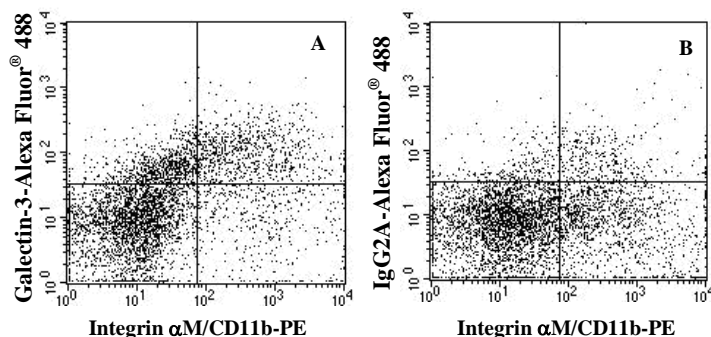
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

## Intended Use

Designed to quantitatively determine the percentage of cells bearing Galectin-3 within a population and qualitatively determine the density of Galectin-3 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 purified, *E. coli*-derived, recombinant mouse Galectin-3 (rmGalectin-3; aa 1 - 264; Accession # P16110). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to Alexa Fluor® 488 fluorochrome. Cell surface expression of Galectin-3 is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 515 - 545 nm.



Mouse splenocytes were stained with either A) Alexa Fluor® 488-conjugated anti-mouse Galectin-3 (Catalog # FAB1197G or B) Alexa Fluor® 488-conjugated isotype control (Catalog # IC006G) and PE-conjugated anti-mouse Integrin αM/CD11b (Catalog # FAB1124P).

## Background Information

Galectin-3, also known as Mac2, L29, CBP35, and εBP, is a chimera galectin that has a tendency to dimerize. Besides the soluble protein, alternatively spliced forms of chicken Galectin-3 containing a transmembrane spanning domain and a leucine zipper motif have been reported. Galectin-3 is expressed in tumor cells, macrophages, activated T cells, osteoclasts, epithelial cells, and fibroblasts. It binds various matrix glycoproteins, including laminin, fibronectin, LAMPS, 90K/Mac2BP, MP20, and CEA. Galectin-3 promotes cell growth and proliferation for many cell types. Galectin-3 acts intracellularly to prevent apoptosis. Depending on the cell types, Galectin-3 exhibits pro- or anti-adhesive properties. Galectin-3 has pro-inflammatory activities *in vitro* and *in vivo*. It induces pro-inflammatory, and inhibits Th2 type, cytokine production. Galectin-3 chemoattracts monocytes and macrophages. It activates and degranulates basophils and mast cells. Elevated circulating levels of Galectin-3 have been shown to correlate with the malignant potential of several types of cancer, suggesting that Galectin-3 is also involved in tumor growth and metastasis. Human and mouse Galectin-3 shares approximately 80% amino acid sequence similarity.<sup>1-4</sup>

## References

- Rabinovich, A. *et al.* (2002) Trends Immunol. **23**:313.
- Rabinovich, A. *et al.* (2002) J. Leukoc. Biol. **71**:741.
- Hughes, R.C. (2001) Biochimie **83**:667.
- Gorski, J.P. *et al.* (2002) J. Biol. Chem. **277**:18840.

## 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/10<sup>5</sup> cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
- After blocking, 5 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 Mouse 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 Alexa Fluor® 488-labeled rat 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.

## Legal

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