Monoclonal Anti-mouse LRRC32-Alexa Fluor® 488

Catalog Number: FAB62291G
Lot Number: ACXY01
100 Tests

Reagents Provided

Alexa Fluor® 488-conjugated rat monoclonal anti-mouse LRRC32: Supplied as 10 μg of antibody in 0.5 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 725226

Isotype: rat IgG_1

Reagents Not Provided

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

Storage

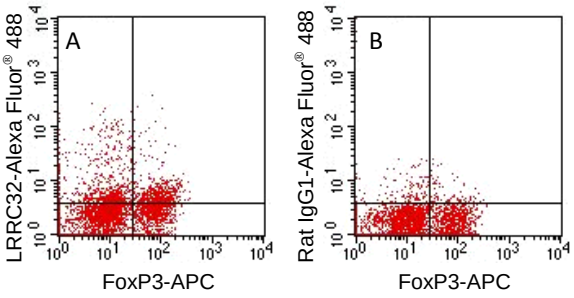
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing LRRC32 within a population and qualitatively determine the density of LRRC32 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 rat immunized with purified, CHO cell-derived, recombinant mouse LRRC32 (rmLRRC32; aa 18-628; Accession # NP001106850). The IgG fraction of the tissue culture supernatant was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to Alexa Fluor® 488 fluorochrome. Cell surface expression of LRRC32/GARP 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 stimulated with anti-CD3/anti-CD28, recombinant mouse IL-2, and TGF-β for 3 days to induce regulatory T cell differentiation. Cells were then stained with PerCP-conjugated CD4 (Catalog # FAB554C), APC-conjugated FoxP3, and A) Alexa Fluor® 488-conjugated LRRC32 (Catalog # FAB62291G), or B) Alexa Fluor® 488-conjugated isotype control (Catalog # IC005G). Dot plots are gated on $CD4^+$ T cells.

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.

Background Information

Leucine-rich repeat protein 32 (LRRC32), also known as GARP (glycoprotein A repetitions predominant), is an 80 kDa type I transmembrane glycoprotein.[1] Mature mouse LRRC32 consists of a 608 amino acid (aa) extracellular domain (ECD) that contains 22 leucine-rich repeats, a 21 aa transmembrane segment, and a 14 aa cytoplasmic domain.[2-4] Within the ECD, mouse LRRC32 shares 80 and 94% aa sequence identity with human and rat LRRC32, respectively. LRRC32 is widely expressed during embryogenesis and on adult platelets.[4, 5] Among T cells, it is selectively expressed on activated $FoxP3^+$ regulatory T cells (Treg).[6-10] LRRC32 expression promotes the acquisition of a Treg phenotype including reduced cellular proliferation, reduced cytokine secretion, and the capacity to suppress the proliferation of naïve T cells.[6-8] LRRC32 binds directly to the TGF-β latency associated peptide (LAP) and tethers latent TGF-β on the surface of activated Treg cells.[9, 10] The presentation of TGF-β on Tregs contributes to their ability to suppress naïve T cell proliferation.[11]

References:

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2. Ollendorff, V. *et al*. (1994) Cell Growth Differ. **5**:213.
3. Bella, J. *et al*. (2008) Cell Mol Life Sci. **65**:2307.
4. Roubin, R. *et al*. (1996) Int. J. Dev. Biol. **40**:545.
5. Macaulay, I.C. *et al*. (2007) Blood **109**:3260.
6. Wang, R. *et al*. (2008) PloS ONE **3**:e2705.
7. Wang, R. *et al*. (2009) Proc. Natl. Acad. Sci. USA **106**:13439.
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11. Vignali, D.A. *et al*. (2008) Nat. Rev. Immunol. **8**:523.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using mouse Treg cells.

1. Cells may be Fc-blocked with 1 μg of mouse IgG/10^5 cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
2. After blocking, 5 μL of conjugated antibody was added to up to 1 x 10^6 cells and incubated for 30 minutes at room temperature.
3. 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 # FC003).
4. 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_1 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.

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