Polyclonal Anti-human EN-RAGE-Alexa Fluor® 488

Catalog Number: FAB1052G
Lot Number: ACDA01
100 Tests

Reagents Provided

Alexa Fluor® 488-conjugated goat polyclonal anti-human EN-RAGE: Supplied as 10 μg of antibody in 0.5 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Isotype: goat IgG

Reagents Not Provided

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

Storage

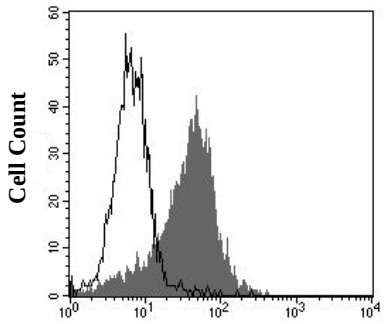
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

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

Product Description

This antibody was produced in goats immunized with purified, *E. coli*-derived, recombinant human EN-RAGE (rhEN-RAGE, aa 1 - 92; Accession # P80511) extracellular domain. Human EN-RAGE specific IgG was purified by human EN-RAGE affinity chromatography. The purified antibody was then conjugated to Alexa Fluor® 488 fluorochrome. Cell surface expression of EN-RAGE 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.



EN-RAGE-Alexa Fluor® 488

Human peripheral blood monocytes were stained with Alexa Fluor® 488-conjugated anti-human EN-RAGE (Catalog # FAB1052G, filled histogram) or Alexa Fluor® 488-conjugated control antibody (Catalog # IC108G, open histogram).

Background Information

EN-RAGE, also known as S100A12 and Calgranulin C, is a 10 kDa secreted molecule that belongs to the S100 family of calcium binding proteins. EN-RAGE contains two EF hand domains and forms dimers and hexamers in solution. EN-RAGE is overexpressed at sites of inflammation and interacts with RAGE on endothelial cells, lymphocytes, and monocytes to promote inflammatory responses. Human EN-RAGE shares 66% and 70% amino acid sequence identity with bovine and porcine EN-RAGE, respectively; mouse and rat orthologs have not been identified.

Flow Cytometry Validation

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

1. Cells may be Fc-blocked with 1 μg of human 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 Human Lyse Buffer (Catalog # FC002).
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 goat IgG 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