

Polyclonal Anti-mouse CRACC/SLAMF7-Allophycocyanin

Catalog Number: FAB4628A

Lot Number: AAUJ01

100 Tests

Reagents Provided

Allophycocyanin (APC)-conjugated sheep polyclonal anti-mouse CRACC/SLAMF7: Supplied as 10 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Antibody type: sheep IgG

Reagents Not Provided

- PBS (Dulbecco's PBS)
- BSA

Storage

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

Intended Use

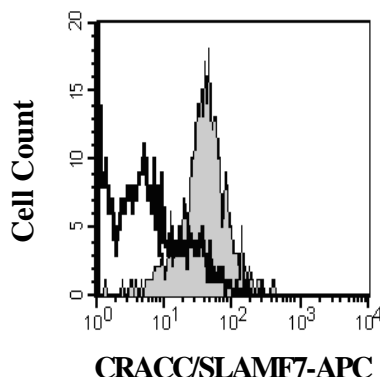
Designed to quantitatively determine the percentage of cells bearing CRACC/SLAMF7 within a population and qualitatively determine the density of CRACC/SLAMF7 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the allophycocyanin-labeled polyclonal antibody, which binds to cells expressing CRACC/SLAMF7. Unbound allophycocyanin-conjugated antibody is then washed from the cells. Cells expressing CRACC/SLAMF7 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of CRACC/SLAMF7. Cell surface expression of CRACC/SLAMF7 is determined by flow cytometry using 620 - 650 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.

Reagent Preparation

Allophycocyanin-conjugated sheep anti-mouse CRACC/SLAMF7: Use as is; no preparation necessary.



NK cells from mouse spleen were stained with APC-conjugated anti-mouse CRACC/SLAMF7 (Catalog # FAB4628A, filled histogram) or isotype control (Catalog # IC016A, open histogram).

Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anticoagulant. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) followed by centrifugation at 500 x g for 5 minutes. 50 µL of packed cells should then be transferred to a 5 mL tube for staining with the polyclonal antibody. Whole blood will require lysis of RBC following the staining procedure.

Cell Cultures: Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA) to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4 x 10⁶ cells/mL and 25 µL of cells (1 x 10⁵) transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from their substrates. Cells that require trypsinization to enable removal from their substrates should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

Sample Staining

- 1) Cells should be Fc-blocked by treatment with 1 µg of mouse IgG/10⁵ cells for 15 minutes at room temperature prior to staining. Do not wash excess blocking IgG from this reaction.
- 2) Transfer 25 µL of the Fc-blocked cells (1 x 10⁵ cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of APC-conjugated CRACC/SLAMF7 reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted CRACC/SLAMF7 reagent by washing the cells twice in 4 mL of the same PBS buffer (*Note: Whole blood will require an RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Whole Blood Lysing Kit, Catalog # WL1000*).
- 6) Finally, resuspend the cells in 200 - 400 µL of PBS buffer for analysis by flow cytometry.
- 7) As a control for this analysis, cells in a separate tube should be treated with APC-labeled sheep IgG antibody.

This procedure may need modification, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

R&D Systems Inc.
1-800-343-7475

Background Information

Produced in sheep immunized with purified, CHO cell-derived, recombinant mouse CRACC extracellular domain (rmCRACC; R&D Systems, Catalog # 4628-SF). Mouse CRACC specific IgG was purified by mouse CRACC affinity chromatography.

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.