Monoclonal Anti-mouse BCMA-Fluorescein

Catalog Number: FAB593F
Lot Number: LKO10
100 tests

Reagent Information

Carboxyfluorescein (CFS)-conjugated rat monoclonal anti-mouse BCMA: Supplied as 50 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

Clone #: 161616

Ig class: rat IgG_1

Additional Reagents Required

- 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

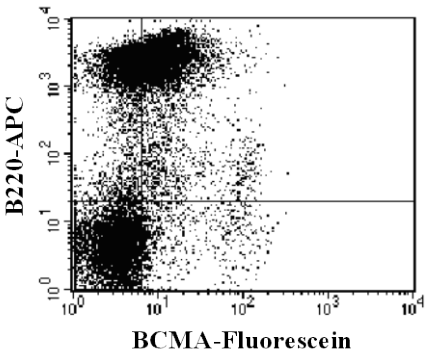
Designed to quantitatively determine the percentage of cells bearing the BCMA cell surface antigen within a population and qualitatively determine the density of the protein on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the fluorescein-labeled monoclonal antibody that binds to the cells expressing BCMA. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells expressing BCMA are fluorescently stained, with the intensity of staining directly proportional to the density of BCMA. Cell surface expression of BCMA is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Fluorescein-conjugated rat anti-mouse BCMA: Use as is; no preparation is necessary.



Mouse splenocytes stained with Fluorescein-conjugated anti-mouse BCMA (Catalog # FAB593F) and APC-conjugated anti-mouse B220 (Catalog # FAB1217A).

Tissue Preparation

Peripheral blood cells and spleen: Whole blood should be collected in tubes containing EDTA or heparin as the anticoagulant. Spleen cells should be first mechanically disaggregated into a single cell suspension. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) by centrifugation at 500 x g for 5 minutes. 50 μL of packed cells are then transferred to a 5 mL tube for staining with the monoclonal. Blood cells 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), as described above, 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 4×10^6 cells/mL and 25 μL of cells (1×10^5) are transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization to enable removal from substrate 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 to be used for staining with the antibody may be first Fc-blocked by treatment 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) Transfer 25 μL of the Fc-blocked cells (up to 1×10^6 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of fluorescein-conjugated anti-mouse BCMA reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unbound anti-BCMA reagent by washing (described above) the cells twice in 4 mL of the same PBS buffer (*note that whole blood will require a RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Mouse Erythrocyte Lysing Kit, Catalog # WL2000*).
6) Resuspend the cells in 200 - 400 μL of PBS buffer for final flow cytometric analysis.
7) As a control for analysis, cells in a separate tube should be treated with fluorescein-labeled rat IgG_1 antibody.

This procedure may need modification, depending upon final utilization.

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

Background Information

The Tumor Necrosis Factor Receptor (TNFR) superfamily of proteins normally consists of type-I molecules with a typical six-cysteine motif linked by intradomain disulphide bonds. Some of the molecules included in this large family include TNFR1 and TNFR2, Fas, DR3, 4 and 5, CD40 and CD30. A recent addition to this family is BCMA (B Cell Maturation Antigen/TNFRSF17) (1, 2). Unlike most other TNFR members, BCMA has two unusual features: it lacks a consensus N-terminal signal sequence suggesting that it may be a type-III rather than a type-I transmembrane protein, and it possesses only one cysteine rich domain instead of the normal three to six. BCMA is a non-glycosylated integral membrane protein that is primarily expressed on mature B cells. The mouse protein sequence consists of 185 amino acids that shares 62% homology with its human counterpart (1, 3). BCMA is expressed on cell surfaces and intracellularly in a perinuclear Golgi-like compartment (4). BCMA acts as a receptor for BAFF (B cell activating factor belonging to the TNF family) and APRIL (a proliferation-inducing ligand) (3, 5). Engagement of BCMA leads to the activation of a variety of intracellular signaling molecules, including JNK, p38 MAPK, NF-κB and Elk-1 (4). The role of BCMA appears to be to deliver a proliferative signal to B cells although BCMA knock-out mice exhibit normal splenic morphology and the ability to generate a normal immune response suggesting some redundancy in the B cell maturation circuitry (6, 7). Over-expression of BAFF has been linked to various autoimmune pathologies including: systemic lupus erythematosus, rheumatoid arthritis, and Sjogren syndrome (8 - 10). In view of the multiple receptors for BAFF (BCMA, TACI and BAFF-R) the ability to monitor the protein expression patterns of these receptors may lead to a better insight into the individual role of these receptors.

References

1. Madry, C. *et al*. (1998) Int. Immunol. **10**:1693.
2. Kwon, B. *et al*. (1999) Curr. Opin. Immunol. **11**:340.
3. Marsters, S. *et al*. (2000) Curr. Biol. **10**:785.
4. Hatzoglou, A. *et al*. (2000) J. Immunol. **165**:1322.
5. Thompson, J.S. *et al*. (2000) J. Exp. Med. **192**:129.
6. Xu, S. and K.P. Lam (2001) Mol. Cell. Biol. **21**:4067.
7. Schiemann, B. *et al*. (2001) Science **293**:2111.
8. Gross, J.A. *et al*. (2000) Nature **404**:995.
9. Groom, J. *et al*. (2002) J. Clin. Invest. **109**:59.
10. Zhang, J. *et al*. (2001) J. Immunol. **166**:6.

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.