

Reagents Provided

Phycoerythrin (PE)-conjugated mouse monoclonal anti-human CD27/TNFRSF7: Supplied as 50 µg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: 57703

Isotype: mouse IgG₁

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 CD27/TNFRSF7 within a population and qualitatively determine the density of CD27/TNFRSF7 on cell surfaces by flow cytometry.

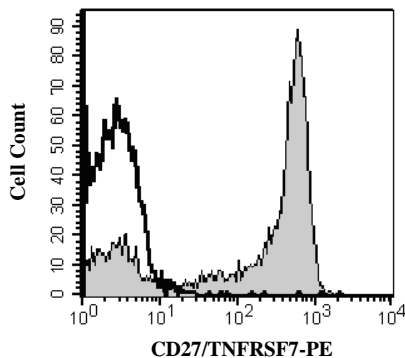
Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled monoclonal antibody, which binds to cells expressing CD27/TNFRSF7. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing CD27/TNFRSF7 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of CD27/TNFRSF7. Cell surface expression of CD27/TNFRSF7 is determined by flow cytometry using 488 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 565 - 605 nm.


Reagent Preparation

Phycoerythrin-conjugated mouse anti-human

CD27/TNFRSF7: Use as is; no preparation necessary.



Human lymphocytes were stained with PE-conjugated anti-human CD27/TNFRSF7 (Catalog # FAB382P, filled histogram) or isotype control (Catalog # IC002P, open histogram).

 フナコシ株式会社

試薬に関して: Tel. 03-5684-1620 / Fax 03-5684-1775

e-mail: reagent@funakoshi.co.jp

2008/03/13

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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 are transferred to a 5 mL tube for staining with the monoclonal 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 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 should be Fc-blocked by treatment with 1 µg of human 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 PE-conjugated CD27/TNFRSF7 reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted CD27/TNFRSF7 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 analysis, cells in a separate tube should be treated with PE-labeled mouse IgG₁ antibody.

This procedure may need modification, depending upon final utilization.

Background Information

Human CD27 is a lymphocyte-specific member of the TNF receptor superfamily. CD27 is expressed on a subset of human thymocytes and on the majority of mature T cells. CD27 expression is up-regulated after TCR stimulation. Within the CD4⁺ compartment, it is preferentially expressed on CD45RA⁺ cells. In contrast, it is preferentially expressed on CD45RO⁺ cells in the CD8⁺ compartment. CD27 also appears to be a potential marker for memory B cells. It exists as both a disulfide-linked dimer on the cell surface and as a soluble protein found in serum. Human CD27 is a 260 amino acid (aa) protein with a 20 aa signal sequence, a 173 aa extracellular domain, a 20 aa transmembrane domain, and a 47 aa cytoplasmic domain. The ligand for CD27 is CD70. CD70 is expressed on thymic stromal cells and a small subset of activated T cells. Additionally, a subset of activated B cells expresses CD70. The CD27/CD70 interaction appears to be a weak co-stimulatory pathway involved in T cell and B cell immune response. CD27/CD70 interactions may be more involved in controlling the expansion phase of an immune response. This would be in contrast to B7/CD28 interactions, which are important for the activation phase of immune responses (Camerini, D. *et al.*, 1991, J. Immunol. **147**:3165 - 3169; Loenen, W.A. *et al.*, 1992 J. Immunol. **149**:3937 - 3943; Lens, S.M.A. *et al.*, 1998, Sem. Immunol. **10**:491 - 499).

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.