Monoclonal Anti-mouse TER-119-Allophycocyanin

Catalog Number: FAB1125A
Lot Number: LOK02
100 Tests

Reagent Information

Allophycocyanin (APC)-conjugated monoclonal anti-mouse TER-119: Supplied as 10 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: TER-119

Ig class: rat IgG_{2B}

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

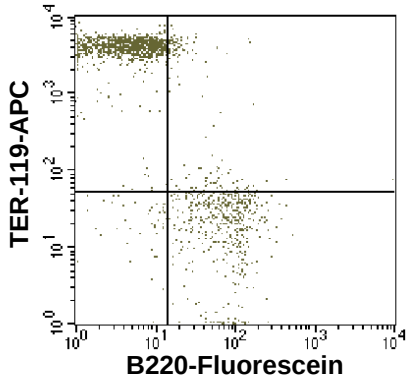
Designed to quantitatively determine the percentage of cells bearing the mouse TER-119 cell surface antigen within a population and qualitatively determine its density on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the APC-labeled monoclonal antibody that binds to cells expressing the mouse TER-119 antigen. Unbound APC-conjugated antibody is then washed from the cells. Cells expressing TER-119 are fluorescently stained, with the intensity of staining directly proportional to the density of TER-119 expression. Cell surface expression of the TER-119 antigen is determined by flow cytometric analysis 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

APC-conjugated rat anti-mouse TER-119: Use as is; no preparation is necessary.



BALB/c mouse bone marrow stained with CFS-conjugated B220 (Catalog # FAB1217F) and APC-conjugated TER-119 (Catalog # FAB1125A).

Sample Preparation

Tissues: 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 x 10^6 cells/mL and 25 μL of cells (1 x 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 (1 x 10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of APC-conjugated anti-mouse TER-119 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-TER-119 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) Finally, 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 APC-labeled rat IgG_{2B} antibody.

This procedure may need modification, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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Background Information

The monoclonal antibody TER-119 is isolated from a hybridoma generated using splenocytes from a rat subcutaneously injected with day 14 BALB/c fetal liver cells (1). The TER-119 monoclonal antibody reacts with erythroid cells from the early proerythroblast to mature erythrocyte stages of development (1). The 52 kDa ligand for TER-119 is associated with glycophorin A on erythrocytes (1). TER-119 antibodies are frequently used in combination with other lineage depletion antibodies to enrich for mouse hematopoietic stem cells (2, 3).

References

1. Kina, T. *et al*., 2000, Br. J. Haematol. **109**:280.
2. Ikuta, K. *et al*., 1990, Cell **62**:863.
3. Osawa, M.Y. *et al*., Hematopoietic stem cells. in *Weir's Handbook of Experimental Immunology*, Vol. 2, 5th Edition. Herzenberg, L.A. *et al*. eds. Cambridge, MA.

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.