Monoclonal Anti-human TACE-Phycoerythrin

Catalog Number: FAB9301P
Lot Number: LBI05
100 tests

Reagent Information

Phycoerythrin (PE)-conjugated mouse monoclonal anti-human TACE Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

Clone #: 111633

Ig class: mouse 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 determine the percentage of cells expressing cell surface TACE and the density of this protein on cell surfaces by flow cytometry.

Principle of the Test

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

Reagent Preparation

PE-conjugated mouse anti-human TACE: Use as is; no preparation is necessary.

Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anti-coagulant. 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. Whole 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 for 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 a 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 human 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 PE-conjugated anti-TACE reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-TACE 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 Whole Blood Lysing Kit, Catalog # WL1000).
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 PE-labeled mouse IgG_1 antibody.

This procedure may need to be modified, depending upon final utilization.

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Background Information

The ADAM (A Disintegrin And Metalloprotease) family includes over 40 proteins containing disintegrin-like and metalloprotease-like domains (1, 2). They are also referred to as MDC (Metalloprotease, Disintegrin, Cystein-rich) proteins (3). ADAMs are involved in diverse processes such as development, cell-cell interactions and protein ectodomain shedding (1-3). The full spectrum of biological functions of many ADAMs has yet to be elucidated.

TACE (Tumor necrosis factor -α converting enzyme) is a transmembrane-bound member of the ADAM family (ADAM17/CD156b). In addition to its ability to release the 17 kDa extracellular soluble form of TNF-α (4, 5), TACE also mediates the ectodomain shedding of various membrane cytokines and receptors, including: tumor necrosis factor-related activation-induced cytokine (TRANCE, 6), transforming growth factor-α (7), L-selectin (7), amyloid precursor protein (8), Notch1 receptor (9), HER4 (10), p55 and p75 of the TNF receptor (7, 11), and IL-1R-II (11). It is unclear how TACE recognizes such a variety of substrates since they exhibit both different topologies (type I and type II) as well as different amino acid sequences within their cleavage recognition sites. The active form of TACE has been found to be expressed on all cells examined (4). Stimulation of cells with phorbol ester increases ectodomain shedding, and down regulates the expression of TACE (12). Additional research interest in TACE stems from its α–secretase activity in Alzheimer's disease and the potential to block release of TNF-α during inflammatory diseases such as rheumatoid arthritis, cachexia, and Chron's disease.

References

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Warning: This reagent 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.