



Product Information

MONOCLONAL ANTI-TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE (TdT) FITC CONJUGATE

Clone 8-1 EF

Purified Mouse Immunoglobulin

Product Number **F6523**

Product Description

Monoclonal Anti-Terminal Deoxynucleotidyl Transferase (TdT), clone 8-1 E4, (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes from mice immunized with bovine TdT. The isotype is determined using a double diffusion assay. The product is prepared by conjugation of fluorescein isothiocyanate (FITC) Isomer I with the purified TdT monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound FITC, no free FITC is detectable.

Monoclonal Anti-TdT (clone 8-1 E4) recognizes the intracellular enzyme terminal deoxynucleotidyl transferase, a 58 kDa protein. TdT is a non-template directed DNA polymerase that catalyses the irreversible addition of deoxynucleotides to the 3'-hydroxy groups on single stranded DNA molecules. The physiological function of TdT has not been determined. The enzyme is thought to play a role in the generation of genetic diversity in T and B cell receptor genes. Normal TdT expression is anatomically restricted to the cortical area of the thymus, immature thymocytes, and about 2% of normal bone marrow cells representing primitive stem cells. TdT expression outside these normal categories has been shown to be a consistent feature of certain leukemias, including ALL, T-ALL, some cases of CML, and Pre-B-ALL. TdT is not expressed in the majority of myeloid leukemias, non-Hodgkins lymphomas, or mature lymphoid leukemias.

Reagents

The antibody is provided (50 µg/ml) as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 0.1% sodium azide as a preservative.

Precautions and Disclaimer

Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

Store at 2-8 °C. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure

Direct Immunofluorescent Staining

Reagents and Materials Needed but Not Provided

1. Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN₃.
2. FITC conjugated, isotype-matched, non-specific mouse immunoglobulin (negative control, Product No. F6397).
3. 12 x 75 mm test tubes.
4. Adjustable micropipette.
5. 0.1% TRITON™ X-100 in Diluent.
6. 1% paraformaldehyde in PBS.
7. Centrifuge.
8. Flow cytometer.

Procedure

Positive control cell lines should be included in the procedure whenever possible. Examples of TdT-expressing stable cell lines include NALM, Molt-4, and GM3639A. Normal peripheral blood lymphocytes can be used as negative cell control. A common type of test sample for TdT is bone marrow. Mononuclear cells can be isolated from such samples using standard Ficoll-Hypaque gradient centrifugation. Since TdT is an intracellular antigen, the staining procedure begins with a permeabilization step. When peripheral blood is used, the lysis of erythrocytes and permeabilization of the remaining leucocytes can be achieved in a single step using commercial preparations of lysing reagent containing diethylene glycol. If simultaneous surface immunophenotyping is required, surface immunofluorescence staining and fixation should be performed prior to permeabilization.

1. Dispense 1×10^6 positive/negative control cells and test sample into 7 x 15 mm tubes.
2. Wash cells twice with 2 ml of Diluent.
3. Fix cells in 2 ml of 1% paraformaldehyde/PBS. Incubate tubes for 10 minutes at 4 °C.
4. Centrifuge cells at 500 x g for 5 minutes at 4 °C. Aspirate supernatant.
5. Add 1 ml 0.1 % TRITON X-100/Diluent and incubate at 4 °C for 3 minutes to permeabilize cells.
6. Centrifuge cells at 500 x g for 5 minutes at 4 °C. Aspirate supernatant.
7. Resuspend cells in 100 µl of Diluent.
8. Stain cells with 10 µl of FITC-anti-TdT (Product No. F6253) or the isotype matched negative control (Product No. F6397).
9. Incubate cells for 30 minutes at 4 °C.
10. Wash cells twice with 2 ml of 0.1% TRITON X-100/Diluent.
11. Resuspend cells in 0.5 ml of 1% paraformaldehyde/PBS for analysis on a flow cytometer.

It is advisable to run the appropriate negative control reagent and negative control cells. The Negative control reagent establishes background fluorescence and non-specific staining of antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein. It should be isotype-matched to the antibody reagent, not specific for human cells and of the same concentration. The degree of autofluorescence or negative control reagent fluorescence will vary with the type of cells under study and the sensitivity of the instrument used. Due to the trapping of reagents in permeabilized cells, non-specific background is a potential problem in any intracellular staining protocol. Negative control cells should always be included to establish a fluorescence baseline with confidence.

Product Profile

When assayed by flow cytometric analysis, using 10 µl of the antibody to stain 1×10^6 cells, maximal resolution is obtained between positive Molt-4 cells expressing the TdT antigen and human peripheral blood lymphocytes serving as negative cell controls.

FITC Conjugated Monoclonal Anti-TdT may be used for:

1. Studies in the development and maturation of T and B lymphocytes.
2. Identification of immature lymphoid precursors, acute lymphoblastic leukemias, lymphoblastic lymphomas, and many chronic granulocytic leukemias.

Note: In order to obtain best results in different preparations it is recommended that each individual user determine their optimum working dilutions by titration assay.

References

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