

Product Information

MONOCLONAL ANTI-TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE CLONE 8-1 E4 Purified Mouse Immunoglobulin

Product No. **T3175**
Lot 084H8960

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 by a double diffusion immunoassay.

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 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 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, and 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 product is provided as purified antibody (100 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 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 hazardous and safe handling practices.

Performance

When assayed by flow cytometric analysis, using 5 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.

Uses

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.

In order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

Storage

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

Procedure for Indirect Immunofluorescent Staining Reagents and Materials Needed but Not Supplied

1. Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN₃.
2. Isotype-matched, non-specific mouse immunoglobulin (negative control, Product No. M5284).
3. FITC-conjugated anti-mouse secondary reagent (e.g. Product No. F2883, FITC-Sheep Anti-Mouse IgG, F(ab')₂ fragment) at the recommended working dilution in diluent.
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. 0.1% Triton X-100 in diluent.
7. 1% paraformaldehyde in PBS.
8. Centrifuge.
9. 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 leukocytes 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 10^6 positive/negative control cells and test sample into 7x15mm tubes.
2. Wash cells twice with 2 ml diluent.
3. Fix cells in 2 ml 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. Add 5 µl of purified anti-TdT (T3175) or the isotype matched negative control (M5284) to similar Ig concentration. Mix gently.
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 100 µl of the FITC anti-mouse Ig secondary reagent at the recommended dilution.
12. Incubate cells for 30 minutes at 4 °C.
13. Wash cells twice with 2 ml of 0.1% Triton X-100/diluent.
14. Resuspend cells in 0.5 ml 1%paraformaldehyde /PBS for analysis in the flow cytometer.

Quality Control

It is advisable to run the appropriate negative control reagent and negative control cells. 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.

References

1. Bollum, F., J. Biol. Chem, **237**, 1945 (1962).
2. Collman, M., et al., Proc. Natl. Acad. Sci. USA, **71**, 4404 (1974).
3. Coleman, M., et al., Cancer Res., **36**, 120 (1976).
4. Greenwood, M., et al., J. Clin. Inv., **59**, 889 (1977).
5. McCaffrey, R., et al., Proc. Natl. Acad. Sci. USA, **70**, 521 (1973).
6. Sarin, P., and Gallo, R., J. Biol. Chem., **249**, 8051 (1974).
7. Syrjala, M., et al., Amer. J. Clin. Path., **99**, 298 (1993).

12/98

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications.

Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply.

Please see reverse side of the invoice or packing slip.