# TdT (Clone: DNTP)
# Rabbit Monoclonal Antibody

**PRODUCT INFORMATION:**

**REF**

| | |
|---|---|
| **MR1298** | **6ml Ready to use** |
| **MR1298** | **3ml Ready to use** |
| **MRC1298** | **1ml Concentrated** |
| **MRC1298** | **0.5ml Concentrated** |
| **MRC1298** | **0.1ml Concentrated** |
| **MRH1298** | **6ml Ready to use** |
| **MRH1298** | **3ml Ready to use** |

**PERFORMANCE CHARACTERISTICS:**

**Localization:** Nucleus
**Retrieval Buffer:** Tris-EDTA, pH 9.0
**Incubation:** 30-60 minutes
**Positive control:** Tonsil, Thymus

## INTENDED USE

**For *research* use only**
This antibody is intended for use in qualitatively identifying TdT antigen by light microscopy in formalin-fixed, paraffin-embedded (FFPE) tissue sections using immunohistochemical (IHC) detection methodology. Interpretation of any positive or negative staining must be complemented with the evaluation of proper known controls (Positive and Negative) and must be made within the context of the patient's clinical history and other diagnostic tests. A qualified and trained pathologist must perform the evaluation of the test. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using non-immunologic histochemical stains.

## SUMMARY AND EXPLANATION

Terminal deoxynucleotidyl transferase (TdT) is an unusual deoxynucleotide polymerizing enzyme with a molecular weight of about 58 kDa found normally only in B- and T-cell lymphoblasts/pre-lymphocytes. TdT generates antigen receptor diversity by synthesizing non-germ line elements (N-regions) at the junctions of rearranged Ig heavy chain and T cell receptor gene segments. Rare TdT-positive cells are regularly detected in thymus and bone marrow. Typically, TdT expression in the thymus is very variable from cell to cell since it is rapidly decreased in more mature T-cells. Tdt-positive cells may occasionally be found in tonsils, lymph nodes and extranodal lymphoid tissue. Immunohistochemical detection of TdT has value in classification of malignant lymphomas and acute leukemias, particularly for the identification of pre-B and pre-T acute lymphoblastic leukemia/lymphoblastic lymphoma (ALL/LBL).
TdT also known as Terminal Deoxynucleotidyl transferase, Terminal Addition Enzyme, Terminal Transferase.

## PRINCIPLE OF THE PROCEDURE

The identification of the antigen on the FFPE tissues is carried out using the above-stated antibody. The antigen and antibody complex are visualized using an enzyme-coupled (HRP/AP) secondary antibody with specific binding to the primary antibody, this complex is visualized by the enzymatic activation of the chromogen resulting in a visible reaction production of the antigenic site. Every step involves precise time and optimal temperature and the results are interpreted using a light microscope by a qualified and trained pathologist.

## REAGENT PROVIDED

**Concentrated format:** TdT is affinity purified and diluted in antibody diluent with 1% bovine serum albumin (BSA) and 0.05% of sodium azide ($NaN_3$).
**Recommended dilutions:** 1:50 – 1:100
The antibody dilution and protocol may vary depending on the specimen preparation and specific application. Optimal conditions should be determined by individual laboratories.
**Pre-diluted format:** PathnSitu's ready-to-use antibodies are pre-tittered to optimal staining conditions. Further dilution will affect the efficacy of the antibody and may yield to sub-optimal staining.
**Immunogen:** Synthetic peptide corresponding to residues within aa1-100 of TdT was used as immunogen.
**Host, Isotype:** Rabbit, IgG

## STORAGE AND HANDLING

**Storage Recommendations:** Store at 2-8°C. When stored at the appropriate conditions, the antibody is stable until expiry. Do not use the antibody after the expiration date provided on the vial in any condition.

**RUO**

To ensure proper reagent delivery and stability, replace the dispenser cap after every use and immediately place the vial in an upright position in refrigerated conditions. The contents of the vial should be used within 9 months from the opening of the vial.

## SPECIMEN PREPARATION

**Staining Recommendations:**
Routinely processed, FFPE tissues are suitable for use with this primary antibody, when using PathnSitu's Poly Excel HRP/DAB detection system. The recommended tissue fixative is 10% neutral buffered formalin. Variable results may occur as a result of prolonged fixation or special processes such as decalcification. The thickness of the sections should be 2-5µm. Slides should be stained once the sections are made as the cut sections' antigenicity may diminish over time. Staining known positive and negative controls simultaneously with unknown specimens is recommended.

## PRECAUTIONS

1. This product should be used by qualified and trained professional users only
2. The product contains < 0.1% of sodium azide as a preservative and is not classified as hazardous, refer to MSDS for further details
3. As with any product derived from biological sources, proper handling procedures should be used
4. Do not use reagents after the expiration date
5. Use protective clothing and gloves, while handling reagents
6. All hazardous materials should be disposed of according to local state and federal regulations
7. Avoid microbial contamination of reagents as it may lead to incorrect results

## STAINING PROCEDURE

**Antigen Retrieval Solution:** Use **Tris-EDTA buffer (Cat# PS009)** as an antigen retrieval solution.
**Heat Retrieval Method**: Retrieve sections under steam pressure for 15 minutes using PathnSitu's MERS (Multi Epitope Retrieval System) for optimal retrieval of the epitopes, allow solution to cool at room temperature, transfer the tissue sections/slides to the distilled water before the primary antibody application.
**Primary Antibody:** Cover the tissue sections with primary antibody and incubate for 30-60 min at room temperature when used PathnSitu's PolyExcel Detection System.
**Detection System:** Refer to PathnSitu's PolyExcel HRP/ DAB detection system protocol for optimal staining results.

## QUALITY CONTROL

The recommended positive tissue control for TdT is Thymus and Thymoma. A positive and negative tissue controls must be run with every staining procedure performed to monitor the correct performance of processed tissue and test reagents. A negative tissue control provides an indication of non-specific background staining. If the results are not expected in positive and negative controls the test must be considered invalid and the entire procedure must be cross-verified. The individual laboratory must establish their own quality control to validate the process and antibody when opening a vial.

## INTERPRETATION OF RESULTS

TdT stains the Nucleus. A qualified experienced/trained pathologist must interpret the results in the patient's sample along with the positive and negative controls.

## ANALYTIC PERFORMANCE CHARACTERISTICS

1. Heat the paraffin-embedded tissue slides for a suitable duration at an appropriate temperature to promote tissue adhesion.

Note: Use positively charged coated slides (Cat no.: PS-011-72) for better adherence.

2. Deparaffinize the slides using xylene (preferably 3 changes with 5min each) to clear the paraffin wax present on and around the tissue.
3. Rehydrate the slides in graded alcohols (100%, 70%, and 50%) for 3 min each and in distilled water (preferably 2 changes with 2 min each) respectively.
4. Immerse the slides in 1X retrieval buffer (preferable Cat No.: PS009) and subject them to Heat-induced epitope retrieval by using a multi-epitope retrieval system (MERS-i) to unmask the epitopes.



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5. Proceed further by using Poly Excel DAB Detection system (preferably Cat no: PEH002 or OSH001) kit components like Poly Excel Peroxidase Block to inactivate or block the non-specific binding firstly.
6. Apply the primary antibody specific to the target antigen. Incubate slides with the primary antibody for a suitable duration at an appropriate temperature as mentioned in the datasheet.
7. Rinse the slides to remove unbound primary antibody using wash buffer (preferably Cat no: PS006)
8. Apply the secondary antibody (preferably Poly Excel Poly HRP- Anti-Mouse/Anti-Rabbit Cat no: PEH002 or OSH001) conjugated to an enzyme that recognizes the primary antibody. Incubate slides with the secondary antibody for a suitable duration at an appropriate temperature.
9. Rinse the slides to remove unbound secondary antibodies using wash buffer (Preferably Immunowash buffer Cat no: PS006)
10. Apply a substrate, PolyExcel Stunn DAB Chromogen for enzyme-conjugated secondary antibody for a suitable duration.
11. Counter-stain the tissue section to visualize the expression in specific structures or cell types.
12. Dehydrate slides through graded alcohols (70%,90%, 100%,100%), clear the slides in Xylene (preferably 3 changes with 2min each) and mount the slides with an appropriate mounting medium.
13. Visualize the stained slides under the microscope.

The antibody consistently exhibited specific and sensitive staining across various positive and negative tissue controls, including Thymus, Thymoma, and DLBCL tissue samples with nuclear staining. This specificity and sensitivity were validated through inter-run, intra-run, and lot-based studies. The stability of the antibody which was determined using real-time or accelerated methods extends until the expiration date indicated on the product labels.

## TROUBLESHOOTING

1. Follow the antibody-specific protocol recommendations according to the datasheet provided
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, tissue processing, antibody freezing and thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, antibody trapping or inaccurate results
3. Do not allow the section to dry out during the entire IHC process
4. Excessive or incomplete counterstaining may compromise the interpretation of the results
5. If unusual results occur, contact PathnSitu's Technical Support at +91-40-2701 5544 or E-mail:techsupport@pathnsitu.com

## LIMITATIONS AND WARRANTY

1. Authorized and skilled/trained personnel only may use the product.
2. The clinical interpretation of any test results should be evaluated within the context of the patient's medical history and other diagnostic test results.
3. A qualified trained pathologist must perform the evaluation of the test results.
4. The product comes with no warranties beyond the provided description
5. Use appropriate volume/concentration to cover entire tissue sections and optimum conditions to avoid false positive and negative results.
6. Use appropriate/recommended buffer/instruments/all consumables with appropriate incubation timings to obtain optimal results.
7. Always recommend using known positive and negative controls to evaluate the test result.
8. Unexpected reactions may occur in untested tissues due to tissue component variability.
9. False positive results can arise from no stringent washing practices and other contributing factors.
10. In instances where localization differs from the specifications outlined in the datasheet, clinical coordination or prompt technical support is advised.
11. Maintain recommended storage conditions.
12. Refer entire data sheet to know any further limitations about the product.
13. No warranties whether expressed or implied, extend beyond the description.
14. PathnSitu is not liable for property damage, personal injury, time or effort or economic loss caused by this product.

## BIBLIOGRAPHY


1. Penninger JM, *et al.*: *Nat Immunol*2001, 2:389-396
2. Kurtin PJ, *et al.*: *Hum Pathol*1985, 16:353-365


**RUO**

## EXPLANATION OF SYMBOLS

LOT **Lot number / Batch number**

**Expiry**

**RUO** ***Research* use only**

**Storage limitation**

**Date of manufacture**

REF **Catalogue number**

**Manufacturer address**




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