

CD4 (Clone: TYP) Rabbit Monoclonal Antibody

PRODUCT INFORMATION:

REF	
MR1322	6ml Ready to use
MR1322	3ml Ready to use
MRC1322	1ml Concentrated
MRC1322	0.5ml Concentrated
MRC1322	0.1ml Concentrated
MRH1322	6ml Ready to use
MRH1322	3ml Ready to use

PERFORMANCE CHARACTERISTICS:

Localization: Membrane
Retrieval Buffer: Tris-EDTA, pH 9.0
Incubation: 30-60 minutes
Positive control: Tonsil, T cell lymphoma

INTENDED USE

For research use only

This antibody is intended for use in qualitatively identifying CD4 antigens 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 an evaluation of the test. This antibody is intended to be used after the primary diagnosis of tumour has been made by conventional histopathology using nonimmunologic histochemical stains

SUMMARY AND EXPLANATION

The CD4 molecule is a glycoprotein, belonging to immunoglobulin superfamily that is associated with HLA class II antigen recognition. It is a co-receptor that assists the T-cell receptor (TCR) with an antigen-presenting cell and also interacts directly with MHC class II molecules on the surface of the antigen-presenting cells using its extracellular domain.

In lymphatic tissues, the CD4+ T cells are seen in large numbers in the parafollicular zone, while scattered cells are found in the germinal centres and mantle zone. CD4 is also demonstrated in hepatic sinusoidal cells, monocytes and monocytes-derived cells but not expressed on B cells and immature thymocytes. Most mature T-cell lymphomas are CD4 positive with the exception of aggressive NK-cell leukaemia and extranodal NK/T-cell lymphomas: subcutaneous panniculitis-like T-cell lymphoma (which is usually CD8 positive), enteropathy-type T-cell lymphoma and hepatosplenic T-cell lymphoma. CD4 is also expressed in histiocytic sarcomas and Langerhans cell histiocytosis as well as in splenic littoral cell haemangioma.

CD4 plays an important role in the classification of lymphocytes in inflammatory lesions and malignant lymphomas.

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. Each and 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: Antibody to CD4 is affinity purified and diluted in antibody diluent with 1% bovine serum albumin (BSA) and 0.05% sodium azide (NaN₃).

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-titrated 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 CD4 residues within aa200-300 of CD4 was used as an 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 expiration date provided on the vial in any condition.

To ensure proper reagent delivery and stability, replace the dispenser cap after every use and immediately place the vial into the refrigerated conditions in an upright position. 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 used 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. Thickness of the sections should be 2-5µm. Slides should be stained once the sections are made as antigenicity of the cut sections may diminish over a period of time. It is recommended to stain known positive and negative controls simultaneously with unknown specimens.

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 prior to the primary antibody application.

Primary Antibody: Cover the tissue sections with primary antibody and incubate for 30-60 min at room temperature when using 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 CD4 is Tonsil, T cell lymphoma, Lymph node and Appendix. A positive and negative tissue control 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 its own quality control to validate the process and antibody when opening a vial.

INTERPRETATION OF RESULTS

CD4 stains the Membrane. A qualified experienced/trained pathologist must interpret the results in the patient's sample along with the positive and negative controls.

PERFORMANCE CHARACTERISTICS

PathnSitu products will undergo a thorough quality control check before it is released to the market. The antibody showed consistent specific and sensitive staining on the multiple positive tissue controls tested, by inter-run, intra run and lot-based studies. The antibody is stable for the expiry mentioned on the labels which is determined by real-time or accelerated methods.

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 artefacts, 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

Authorized and skilled/trained personnel only may use the product. The clinical interpretation of any test results should be evaluated within the context of the patient's medical history and other diagnostic test results. A qualified trained pathologist must perform the evaluation of the test results. There are no warranties, expressed or implied, which extend beyond the description. PathnSitu is not liable for property damage, personal injury, time or effort or economic loss caused by this product.

BIBLIOGRAPHY

1. Karandikar NJ, et.al, Am J Clin Pathol. 2002 May;117(5):819-25.
2. Beaty MW, et.al, Am J Surg Pathol. 2001 Sep;25(9):1111-20.

EXPLANATION OF SYMBOLS



Lot number / Batch number



Expiry

RUO *Research use only*



Storage limitation



Date of manufacture



Catalogue number