

**C4D (Clone: EP272)
Rabbit Monoclonal Antibody**

PRODUCT INFORMATION:	PERFORMANCE CHARACTERISTICS:
MR1208 6ml Ready to use	Localization: Cytoplasm
MR1208 3ml Ready to use	Retrieval Buffer: Tris-EDTA, pH 9.0
MRC1208 1ml Concentrated	Incubation: 60 minutes
MRC1208 0.5ml Concentrated	Positive control: Rejected Kidney
MRC1208 0.1ml Concentrated	(antibody-Mediated)
MRH1208 6ml Ready to use	
MRH1208 3ml Ready to use	

INTENDED USE

For research use only
This antibody is intended for use in qualitatively identify C4D 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 evaluation of the test. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains.

SUMMARY AND EXPLANATION

C4D is the degradation product of the activated complement factor C4, following activation and degradation of the C4 molecule, thioester groups are exposed which allow transient, covalent binding of the degradation product C4D to endothelial cell surfaces and extracellular matrix components of vascular basement membranes near the sites of C4 activation. C4D is also found in intracytoplasmic vacuoles of endothelial cells. Covalent binding renders C4D a stable molecule that can easily be detected by immunohistochemistry. Feucht et al. were the first to demonstrate the occurrence of capillary C4D staining in kidney allografts and its association with inferior graft outcomes, and later confirmed by other studies. It was demonstrated that patients with suspected antibody-mediated injury in the renal graft had a linear C4D staining pattern in peritubular capillaries and that the presence of C4D was associated with impaired graft function. Except kidney allografts, little is known currently about C4D accumulation in other solid organ allografts. Preliminary data suggest that heart allografts are comparable with kidney transplants. C4D was found in early post transplant endomyocardial biopsies and was associated with poor graft survival. In dysfunctioning lung transplants, C4D could be detected in septal capillaries. C4D has also been found in liver allografts carrying a diagnosis of antibody-mediated rejection.

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 is 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 to 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 C4D is affinity purified and diluted in antibody diluent with 1% bovine serum albumin (BSA) and 0.05% of 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 laboratory.
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: Prokaryotic recombinant protein corresponding to the C4D protein.
Host, Isotype: Rabbit, IgG

STORAGE AND HANDLING

Storage Recommendations: Store at 2-8°C. When stored at the appropriate

DS-MR1208-A

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 preservative and is not classified hazardous, refer 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 expiration date
5. Use protective clothing and gloves, while handling reagents
6. All hazardous materials should be disposed 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 antigen retrieval solution.
Heat Retrieval Method: Retrieve sections under steam pressure for 20 minutes using PathnSitu's MERS (Multi Epitope Retrieval System) for optimal retrieval of the epitopes, allow solution to cool at the 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 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 C4D is Rejected Kidney (antibody mediated). A positive and negative tissue control must be run with every staining procedure performed for monitoring the correct performance of processed tissue and test reagents. A negative tissue controls provide 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 entire procedure must be cross verified. Individual laboratory must establish their own quality control to validate the process and antibody when opened a vial.

INTERPRETATION OF RESULTS

C4D stains the Cytoplasm. 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 data sheet 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

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 on economic loss caused by this product.

BIBLIOGRAPHY

1. Regele H, et al; J Am Soc Nephrol 2002, 13:2371–2380
2. Nickleit V, et al; Nephrol Dial Transplant. 2003,18(11):2232-9
3. Feucht HE, et al; Clin Exp Immunol 1991, 86:464–470
4. Feucht HE, et al; Kidney Int 1993, 43:1333–1338
5. Böhmig GA, et al; J Am Soc Nephrol. 2002,13(4):1091-9
6. Corrêa RR, et al; Clin Dev Immunol. 2013, 2013:678180.

C4D, EP272 antibody has been created by Epitomics Inc., using Epitomics proprietary rabbit monoclonal antibody technology covered under Patent No.'s 5,675,063 and 7,402,409.

EXPLANATION OF SYMBOLS

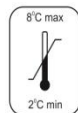
LOT- Lot number / Batch number



- Expiry

RUO

Research use only



Storage limitation