

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

**PRODUCT INFORMATION:**

REF	
MR1208	6ml Ready to use
MR1208	3ml Ready to use
MRC1208	1ml Concentrated
MRC1208	0.5ml Concentrated
MRC1208	0.1ml Concentrated
MRH1208	6ml Ready to use
MRH1208	3ml Ready to use

**PERFORMANCE CHARACTERISTICS:**

**Localization:** Cytoplasm  
**Retrieval Buffer:** Tris-EDTA, pH 9.0  
**Incubation:** 60 minutes  
**Positive control:** Rejected Kidney (antibody-Mediated)

**INTENDED USE**

**For research use only**

This antibody is intended for use in qualitatively identifying C4D antigen by light microscopy in formalin-fixed, paraffin-embedded (FFPE) tissue sections using immunohistochemical (IHC) detection methodology. Any observed staining or its absence should be reviewed alongside appropriate positive and negative controls. Interpretation must be carried out by qualified personnel trained in histological and molecular techniques. This product is for research use only and not intended for diagnostic or therapeutic use.

**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 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 product 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:** C4D is affinity purified and diluted in antibody diluent with 1% bovine serum albumin (BSA) and 0.05% of sodium azide (NaN<sub>3</sub>).

**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:** 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 conditions, the antibody is stable until expiry. Do not use the antibody after the

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 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 20 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 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 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**

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.

**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.
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 Rejected Kidney, Glomerular Sclerosis (Allograft Kidney), and brain tissue samples with cytoplasm 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](mailto: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. 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 number / Batch number



Expiry



Research use only



Storage limitation



Date of manufacture



Catalogue number



Manufacturer address