

## C4d (RM)

Concentrated and Prediluted Rabbit Monoclonal Antibody  
901-3134-080917

**BIOCARE**  
M E D I C A L

<b>Catalog Number:</b>	<b>ACI 3134 A, B</b>	<b>API 3134 AA</b>
<b>Description:</b>	0.1, 0.5 ml, concentrated	6.0 ml, prediluted
<b>Dilution:</b>	1:100	Ready-to-use
<b>Diluent:</b>	Renoir Red	N/A

### Intended Use:

For In Vitro Diagnostic Use

C4d (RM) [A24-T] is a rabbit monoclonal antibody that is intended for laboratory use in the qualitative identification of C4d protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

### Summary and Explanation:

C4d is a stable split product remnant of classical complement activation which becomes covalently bound to endothelium and basement membrane (1). Capillary deposition of complement C4d has been suggested to be a valuable marker for humoral rejection and endothelial C4d deposition in kidney allograft has been associated with inferior graft outcome (1-3). The detection of C4d in formalin-fixed, paraffinembedded tissue has been documented to be valuable in the evaluation of various inflammatory diseases (4). Membranous nephropathy (MN) is the most common cause of nephrotic syndrome in adults and C4d immunohistochemical staining has been shown to be a very useful tool for MN (5).

In the past, most publications have used polyclonal antibodies against C4d. However, polyclonal antibodies are known to vary from batch to batch. C4d [A24-T] is a rabbit monoclonal antibody suitable for formalin-fixed paraffin-embedded tissues.

### Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, an enzyme labeled polymer is added to bind to the primary antibody. The detection of the bound antibody is evidenced by a colorimetric reaction.

**Source:** Rabbit monoclonal

**Species Reactivity:** Human; others not tested

**Clone:** A24-T

**Isotype:** IgG

**Total Protein Concentration:** ~10 mg/ml. Call for lot specific Ig concentration.

**Epitope/Antigen:** Internal sequence of human C4

**Cellular Localization:** Basement membrane/cytoplasm

**Positive Tissue Control:** Renal allograft tissue

### Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

**Supplied As:** Buffer with protein carrier and preservative

### Storage and Stability:

Store at 2°C to 8°C. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

### Protocol Recommendations:

#### Peroxide Block:

Block for 5 minutes with Biocare's Peroxidized 1.

### Protocol Recommendations Cont'd:

**Pretreatment:** Perform heat retrieval using Biocare's Diva Decloaker. Refer to the Diva Decloaker product data sheet for specific instructions.

**Protein Block (Optional):** Incubate for 5-10 minutes at RT with Biocare's Background Punisher.

**Primary Antibody:** Incubate for 30 minutes at RT.

**Probe:** N/A

**Polymer:** Incubate for 30 minutes at RT with a secondary-conjugated polymer.

#### Chromogen:

Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Biocare's Warp Red.

#### Counterstain:

Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

#### Technical Note:

This antibody has been standardized with Biocare's MACH 4 detection system. Use TBS buffer for washing steps.

#### Limitations:

The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

#### Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA ([www.clsi.org](http://www.clsi.org)). 2011

#### Precautions:

1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN<sub>3</sub>) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (6)

2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water. (7)

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### Precautions Cont'd:

3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at <http://biocare.net>.

### Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

### References:

1. Troxell ML, *et al.* Comparison of C4d immunostaining methods in renal allograft biopsies. Clin J Am Soc Nephrol. 2006 May; 1(3):583-91.
2. Regele H, *et al.* Endothelial C4d deposition is associated with inferior kidney allograft outcome independently of cellular rejection. Nephrol Dial Transplant. 2001 Oct; 16(10):2058-66.
3. Böhmig GA, *et al.* Capillary C4d deposition in kidney allografts: a specific marker of alloantibody-dependent graft injury. J Am Soc Nephrol. 2002 Apr; 13(4):1091-9.
4. Magro CM, Dyrsen ME. The use of C3d and C4d immunohistochemistry on formalin-fixed tissue as a diagnostic adjunct in the assessment of inflammatory skin disease. J Am Acad Dermatol. 2008 Nov; 59(5):822-33.
5. Espinosa-Hernández M, *et al.* C4d as a diagnostic tool in membranous nephropathy. Nefrologia. 2012 May 14; 32(3):295-9.
6. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
7. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.