

C4d

Mouse Monoclonal Antibody

MM11-6

MM11-10

Document #: IFU-MM11-C4d
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Immunogen	Clone	Species	Isotype	Primary Antibody Diluent
A recombinant fragment specific to Complement 4d	C4D204	Mouse	Mouse IgG1	NA

Lot Specific Ig concentration available upon request.

Catalog	Description
MM11-6	6 mL Ready To Use antibody for use with StatLab Medical Ultra High Def Polymer - Two Step Detection System
MM11-10	10 mL Barcoded Ready To Use antibody for use with StatLab Medical Ultra High Def Polymer Detection System on the automated IHC 360 System

Intended Use

For In Vitro Diagnostic Use. This product is intended for qualitative immunohistochemistry with normal and neoplastic formalin-fixed, paraffin-embedded tissue sections to be viewed by light microscopy. Clinical interpretation of staining results should be accompanied by histological studies with proper controls. Patients' clinical histories and other relevant diagnostic tests should be utilized by a qualified person(s) when evaluating and interpreting results.

Summary and Explanation

Complement 4d (C4d) is the most clinically used marker for humoral rejection. It is a degradation product of the activated complement factor C4b. Complement 4d is typically initiated by binding of antibodies to specific target molecules. Following activation and degradation of the C4 molecule, thio-ester groups are exposed, which allow transient, covalent binding of the degradation product Complement 4d to endothelial cell surfaces and extracellular matrix components of vascular basement membranes near the sites of C4 activation. Complement 4d is also found in intracytoplasmic vacuoles of endothelial cells. Covalent binding renders C4d a stable molecule that can easily be detected by immunohistochemistry.

Format

This antibody is affinity purified and diluted in 10 mM Phosphate buffered saline (PBS), pH 7.5 containing 1% bovine serum albumin (BSA) and 0.05%

sodium azide (NaN₃) as a preservative.

Principles of the Procedures

Antigen detection by immunohistochemistry (IHC) is a two-step process involving first, the binding of a primary antibody to the antigen of interest, and second, the detection of bound antibody by a chromogen. The primary antibody may be used in IHC using manual techniques or using automated IHC Staining Systems.

Dilution of Primary Antibody

Ready-to-Use antibodies have been optimized for use with the recommended Polymer Detection System and should not require further dilution. Further dilution may result in loss of sensitivity. The user must validate any such change.

StatLab Concentrated antibodies must be diluted in accordance with the staining procedure when used with the recommended Detection System. Use of any detection methods other than the recommended systems and protocols require validation by the user. Antibody dilutions should be appropriately adjusted and verified according to the detection system used.

Materials Required But Not Provided

All the reagents and materials required for IHC are not provided. Pretreatment reagents, detection systems, control slides, control reagents and other ancillary reagents are available from StatLab. Please refer to our website at: www.StatLab.com

Storage and Handling

Store at 2-8°C. This antibody is suitable for use until expiry date when stored at 2-8°C. do not use product after the expiration date printed on vial. If reagents are stored under a condition other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly. Unused portions of antibody preparation should be discarded after one day.

The presence of precipitate or an unusual odor indicates that the antibody is deteriorating and should not be used.

Positive and negative controls should be run simultaneously with all patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact StatLab IHC Technical Support via Email at: ihctech@statlab.com or call us at (800) 442-3573.

Specimen Collection and Preparation

Tissues fixed in 10% formalin are suitable for use prior to paraffin embedding. Consult references (Kiernan, 1981; Sheehan & Hrapchak, 1980) for further details on specimen preparation.

The user is advised to validate the use of the products with their tissue specimens prepared and handled in accordance with their laboratory practices.

Precautions

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 (NaN₃) 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). 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 in contact with sensitive areas, wash with copious amounts of water. Microbial contamination of reagents may result in an increase in nonspecific staining. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change. The SDS is available upon request.

Treatment of Tissues Prior to Staining

Pretreatment of tissues, if any, should be done as suggested: Place the slides in the recommended Antigen Retrieval Solution using an appropriate retrieval/pressure cooker system. Set the temperature for a 15-minute incubation at "High Pressure". Allow slides to cool down for 20 minutes prior to staining.

Staining Procedure

Refer to the following table for conditions specifically recommended for this antibody. Refer to the StatLab Ultra High Def—Two Step Detection System for guidance on specific staining protocols or other requirements.

Parameter	StatLab Recommendations
Positive Control	Tonsil
Concentrated Dilution	N/A
Pretreatment	EDTA Buffer, pH 8.0
Incubation Time & Temperature	30 min @ RT
Detection System	Ultra High Def Polymer - Two Step Detection System
Tissue Type	FFPE

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). 2011.

Troubleshooting

StatLab Headquarters: 2090 Commerce Drive, McKinney, TX 75069.
 Email our team at ihctech@statlab.com
 Call at (800) 442-3573.

Cellular Localization

Membrane/Cytoplasm

Limitations of the Procedure

Immunohistochemistry is a complex technique involving both histological and immunological detection methods. Tissue processing and handling prior to immunostaining can also cause inconsistent results. Variations in fixation and embedding or the inherent nature of the tissue may cause variations in results (Nadji and Morales, 1983). Endogenous peroxidase activity or pseudoperoxidase activity in erythrocytes and endogenous biotin may cause non-specific staining depending on detection system used. Tissues containing Hepatitis B surface Antigen (HBsAg) may give false positive with horseradish peroxidase systems (Omata et al, 1980). Improper counterstaining and mounting may compromise the interpretation of results.

Performance Characteristics

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, and 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 products manufactured for StatLab. Ultimately, it is the responsibility of the investigator to determine optimal conditions. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

References

