

Anti-C4d (Clone: 10-11)
Mouse monoclonal antibody
Reference: AP10360C



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INTENDED USE AND PRESENTATION:

For *in vitro* diagnostic use.

AP10360C, 0.1ml. Concentrated purified IgG, liquid. Borate buffered saline, pH 8.4 with carrier protein and preservative for stabilisation.

SUMMARY, EXPLANATION AND LIMITATIONS:

This antibody recognises the secreted protein complement component 4d (C4d). The complement 1 complex cleaves complement 4 (C4) to form C4b and C4a. C4b levels are strictly regulated. Single site cleavage of the C4b's alpha chain by Factor I forms iC4b and blocks C3 convertase, inhibiting opsonisation and activation of the classical pathway. This requires C4 binding protein or CR1 as a cofactor. iC4b is further degraded into C4d and C4c. C4d's short half life means that C4d is present in serum at high enough concentrations to make it a useful marker for classical complement activation. C4 has been linked to susceptibility to systemic lupus erythematosus.

Immunohistochemistry (IHC) is a complex technique in which immunological and histological detection methods are combined. In general, the manipulation and processing of tissues before immunostaining, especially different types of tissue fixation and embedding, as well as the nature of the tissues themselves may cause inconsistent results (Nadji and Morales, 1983). Endogenous pseudoperoxidase and peroxidase activity or endogenous biotin and alkaline phosphatase activity can cause non-specific staining results depending on the detection system used. Tissues that contain Hepatitis B surface antigen (HBsAg) can produce false positives when using HRP detection systems (Omata et al, 1980). Insufficient contrast staining and/or improper mounting of the sample may influence the interpretation of results.

Isotype: IgG1

Immunogen: Native, from human plasma.

Staining pattern: Cytoplasmic/Secreted.

The interpretation of the stain results is the full responsibility of the user. Any experimental result must be confirmed by a medically established diagnostic product or procedure.

Positive control: Tissue sample from kidney.

External negative control: Tissue sample homologous to the test sample incubated with an antibody isotype not specific for C4d.

APPLICATIONS:

This antibody is designed for the specific localization of human C4d using IHC techniques in frozen tissue and formalin-fixed, paraffin-embedded tissue sections.

PRODUCT COMPOSITION:

Mouse immunoglobulin IgG1 purified, clone 10-11, prepared by affinity chromatography on Protein A. The preparation contains saline buffer, stabilising and carriers proteins, and sodium azide as a preservative.

METHODS AND PROCEDURE:

Principles of the procedure: The demonstrations of antigens by IHC is a sequential procedure with several steps involving first the application of a specific antibody for the antigen of interest (primary antibody), then a secondary antibody which joins to the first, an enzyme complex, and the addition of a chromogenic substrate. The sample is washed between each step. Enzymatic activation of the chromogenic substrate creates a visible product where the antigen is located. The results are interpreted using a light microscope. The primary antibody can be used both in manual IHC and with automated immunostainers.

Specimen: Paraffin-embedded tissue samples should be used. The antibody is also useful for immunostaining frozen tissue samples.

It has been reported that this antibody works very well on acetone fixed, frozen renal biopsies. Strong staining is observed in the glomeruli and in some cases the peritubular capillaries (Mauillyedi, S. et al. 2002).

Clone 10-11 has given variable results on FFPE sections. It has been observed that pretreatment with 88% formic acid for 20 minutes at room temperature is beneficial (Stoltzner, SE. et al. 2000).

Suggested working dilutions:

IHC-P	1:10 – 1:100
IHC-F	1:100 – 1:500
IF	1:200 – 1:600
ELISA	1:5000 – 1:20000
WB	1:10 – 1:1000

Amplification and development of the immunostaining:

Follow standard procedure and the recommendations given by the manufacturer for the materials used. In the case of using automated immunostainers, use the specified buffers and materials for each instrument.

See our web site at www.gennova-europe.com for detailed protocols ancillary reagents and support products.

REQUIRED MATERIALS BUT NOT SUPPLIED:

All reagents, materials, and laboratory equipment for IHC procedures are not provided with this antibody. This includes adhesive slides and cover slips, positive and negative control tissues, Xylene or adequate substitute, ethanol, distilled H₂O, heat pretreatment equipment (pressure cooker, steamer, microwave), pipettes, Coplin jars, glass jars, moist chamber, histological baths, negative control reagents, counter-staining solution, mounting materials, and microscope.

Buffered solutions for antigen retrieval, enzyme treatments, highly sensitive detection systems, and other auxiliary reagents are available from Genova Scientific.

STORAGE AND STABILITY:

Store at 2-8 °C until the expiration date printed on product label. Do not use after the expiration date. Concentrated antibody can be stored in -20 °C if preferred. Storage in frost-free freezers is not recommended. Avoid repeated freezing and thawing as this may denature the antibody. Should this



Catalog number



Batch code



In Vitro diagnostic medical device



Temperature limitation



Expiration date



Manufacturer



See instruction for use



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product contain a precipitate we recommend microcentrifugation before use. If fresh solutions are required, these must be prepared immediately prior to use, and will be stable for at least one day at room temperature (20-25°C). Unused portion of antibody preparation should be discarded after one day. If the product is stored under different conditions from those stipulated in these technical indications, the new conditions must be verified by the user.

Gennova Scientific guarantees that the product will maintain all of the described characteristics from the production date until the expiration date, as long as the product is stored and used as recommended. No other guarantees are provided. Under no circumstances is Gennova Scientific obliged to cover damages caused by use of this reagent.

TROUBLESHOOTING:

If unusual staining is observed or any other deviations from the expected results, please read these instructions carefully, along with the instructions from the detection system. If this does not solve the problem, please contact Gennova Scientific's technical support department or your local distributor.

PRECAUTIONS:

Use only by qualified personnel.

Use proper protective equipment in order to avoid contact with reagents and samples in the eyes, skin, and mucosal tissues. In case of contact with sensitive areas, immediately flush the affected area with water. Avoid microbial contamination of the reagent, as this may produce nonspecific staining results. This antibody contains sodium azide (NaN₃), used as a stabilising agent, which is not considered to be a hazardous material in the concentration used. Concentration of sodium azide in drainage pipes made of lead or copper can cause the formation of highly explosive metallic azides. In order to avoid this, sodium azide must be disposed of along with a large volume of running water. Material safety data sheet (MSDS) for pure sodium azide is available upon request.

PERFORMANCE CHARACTERISTICS:

Gennova Scientific has performed studies to evaluate the functioning of these antibodies for use with standard detection systems, concluding that the product is both specific and sensitive for the antigen of interest.

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Catalog number



Batch code



In Vitro diagnostic medical device



Temperature limitation



Expiration date



Manufacturer



See instruction for use



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