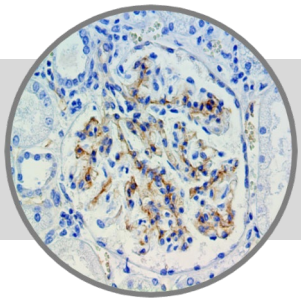
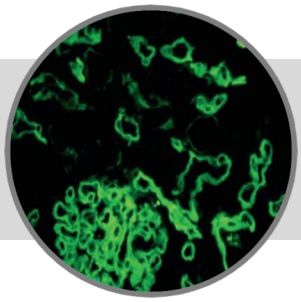
C4d, RMab

Clone: EP272

Rabbit Monoclonal





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Inset: IHC and IF of C4d on a FFPE Kidney Tissue (IHC) and a Frozen Kidney Rejection Tissue (IF)

Intended Use

For Research Use Only.

This antibody is intended for use in Immunohistochemical (IHC) and Immunofluorescence (IF) applications on formalin-fixed paraffin-embedded tissues (FFPE), frozen tissue sections and cell preparations. Interpretation of results should be performed by a qualified medical professional.

* The C4d antibody, clone EP272, has been manufactured using Epitomics RabMab® technology covered under Patent No.'s 5,675,063 and 7,402,409.

Immunogen

Prokaryotic recombinant protein corresponding to the C4d protein.

Summary and Explanation

Complement component 4, or C4, plays a central role in the complement system. C4d is the final proteolytic remnant of deposited C4b on endothelium and remains covalently attached to endothelium for little more than a week. It is easily detectable by Immunohistochemistry.

Anti-C4d combined with anti-C3d can be utilized as a tool for diagnosis of AR (Acute Rejection) and warrant prompt and aggressive anti-rejection treatment. C4d can be detected in peritubular capillaries in both chronic renal allograft rejection as well as hyperacute rejection, acute vascular rejection, acute cellular rejection, and borderline rejection. It has been shown to be a significant predictor of transplant kidney graft survival and is an aid in treating acute rejection.

Antibody Type	Rabbit Monoclonal	**Clone**	EP272
Isotype	IgG	**Reactivity**	Paraffin, Frozen
Localization	Cytoplasmic, Membranous	**Control**	Tonsil, Lymph Node, Spleen, Kidney, Kidney Transplant Rejection
	Species Reactivity	Human, Rat, Mouse	

Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. This product contains <0.1% sodium azide (NaN_3) as a preservative. Ensure proper handling procedures are used with this reagent.
3. Always wear personal protective equipment such as laboratory coat, goggles and gloves when handling reagents.
4. Dispose of unused solution with copious amount of water.
5. Do not ingest reagent. If reagent is ingested, seek medical advice immediately.
6. Avoid contact with eyes. If contact occurs, flush with large quantities of water.
7. Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker or similar).
8. For additional safety information refer to Safety Data Sheet for this product.
9. For complete recommendations for handling biological specimens, please refer to the CDC document, "Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories" (see References in this document).

Presentation

C4d is a rabbit monoclonal antibody derived from cell culture supernatant that is concentrated, dialyzed, filter sterilized and diluted in buffer pH 7.5, containing BSA and sodium azide as a preservative.

Catalog No.	*Antibody Type*	*Suggested Dilution IHC/ IF*	*Volume/Qty*
BSB 2831	Tinto Prediluted	Ready-to-Use*	3.0 mL
BSB 2832	Tinto Prediluted	Ready-to-Use*	7.0 mL
BSB 2833	Tinto Prediluted	Ready-to-Use*	15.0 mL
BSB 2834	Concentrated	1:100 / 1:50	0.1 mL
BSB 2835	Concentrated	1:100 / 1:50	0.5 mL
BSB 2836	Concentrated	1:100 / 1:50	1.0 mL

***Ready-to-Use, for IHC only**

Control Slides Available

Catalog No.	*Quantity*
BSB 2837	5 slides

Storage Store at 2-8°C (Control Slides: Store at 20-25°C)

Stability

This product is stable up to the expiration date on the product label. Do not use after expiration date listed on package label. Temperature fluctuations should be avoided. Store appropriately when not in use, and avoid prolonged exposure to room temperature conditions.

Specimen Preparation

Paraffin sections: The antibody can be used on formalin-fixed paraffin-embedded (FFPE) tissue sections. Ensure tissue undergoes appropriate fixation for best results. Pre-treatment of tissues with heat-induced epitope retrieval (HIER) is recommended using Bio SB ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023), ImmunoDNA Retriever with EDTA (BSB 0030-BSB 0033) or ImmunoDNA Digestor (BSB 0108-0112). See reverse side for complete protocol. Tissue should remain hydrated via use of Bio SB Immuno/DNA Washer solutions (BSB 0029 & BSB 0042).

Frozen sections and cell preparations: The antibody can be used for labeling acetone-fixed frozen sections and acetone-fixed cell preparations.

Staining Procedure

Preparation for Frozen Tissues

1. Embed the specimen in OCT inside a cryostat.
2. Cut sections at 4-5 microns a and mount on a positively charged glass slide such as the Bio SB Hydrophilic Plus Slides (BSB 7028).
4. Air dry at 58- 60 °C for 10 minutes.
5. Fix in acetone 100% or NBF10% for 2-10 minutes.
6. Air dry for another 2 minutes.

Preparation for FFPE Tissues

1. Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positively charged slides such as Bio SB Hydrophilic Plus Slides (BSB 7028).
2. Air dry for 2 hours at 58° C.
3. Deparaffinize, dehydrate and rehydrate tissues.
4. Subject tissues to heat induced epitope retrieval (HIER) using a suitable retrieval solution such as ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023) or EDTA (BSB 0030-BSB 0033).
5. Any of three heating methods may be used:

a. TintoRetriever Pressure Cooker or Equivalent

Place tissues/slides in a staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA, and place on trivet in the pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high. Incubate for 15 minutes. Open and immediately transfer slides to room temperature.

b. TintoRetriever PT Module or Water Bath Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA at 95°-99° C. Incubate for 30-60 minutes.

c. Conventional Steamer Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA in a steamer, cover and steam for 30-60 minutes.

6. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.
7. Wash slides with ImmunoDNA washer or DI water.
8. For manual staining, perform antibody incubation in the dark at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
9. Continue with IHC or IF staining protocol.

Abbreviated Immunohistochemical Protocol

Step	ImmunoDetector AP/HRP	PolyDetector AP/HRP	PolyDetector Plus AP/HRP
Peroxidase/AP Blocker	5 min.	5 min.	5 min
Primary Antibody	30-60 min.	30-60 min.	30-60 min.
1st Step Detection	10 min.	30-45 min.	15 min.
2nd Step Detection	10 min.	Not Applicable	15 min.
Substrate-Chromogen	5-10 min.	5-10 min.	5-10 min.
Counterstain / Coverslip	Varies	Varies	Varies

Abbreviated Immunofluorescence Protocol

Step	Incubation Time
Rinse slides in IF wash buffer	5 min
Apply Antibody	30-60 min.
Rinse with 3 changes of IF wash buffer	3 x 5 min. each
Apply Rabbit FluoroDetector FITC	15 min.
Rinse with 3 changes of IF wash buffer	3 x 5 min. each
Coverslip with FluoroMounter medium	

Mounting Protocols

IHC:

For detailed instructions using biodegradable permanent mounting media such as XyGreen PermaMounter (BSB 0169-0174) or organic solvent-based resin such as PermaMounter (BSB 0094-0097), refer to PI0174 or PI0097.

IF:

1. Bring FluoroMounter or FluoroMounter with DAPI to room temperature.
2. Rinse slides with distilled or deionized water.
3. Remove excess of water from slides before laying them flat in the dark.
4. Turn the media bottle upside down before opening the dropper bottle.
5. Apply 1-3 drops of FluoroMounter to each slide making sure the specimen is covered.
6. Incubate 3-5 minutes at room temperature in the dark.
7. Coverslip.
8. Observe under a fluorescent microscope using the appropriate filters.
9. The slides are recommended to be stored at 2-8 ^{0}C in the dark.

Product Limitations

Due to inherent variability present in immunohistochemical and immunofluorescent procedures (including fixation time of tissues, dilution factor of antibody, retrieval and detection system used and incubation time), optimal performance should be established using positive and negative controls. Results should be interpreted by a qualified medical professional.

References

1. Liu C, et al. Arthritis Rheum. 2005; 52(10):2087-99.
2. Jianghua C, et al. Clin Transplant. 2005 Dec; 19(6):785-91.
3. Kayler LK, et al. Transplantation. 2008 March; 85(6):813-20.
4. Seemayer CA, et al. Nephrol Dial Transplant. 2007 Feb; 22(2):568-76.
5. Nadasdy GM, et al. Hum Pathol. 2005 Nov; 36(11):1178-85.
6. Bouron-Dal Soglio D, et al. Hum Pathol. 2008 Jul; 39(7):1103-10
7. U.S. Department of Health and Human Services: Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. Supplement / Vol. 61, January 6, 2012.

Symbol Key / Légende des symboles/Erläuterung der Symbole

Storage Temperature Limites de température Zulässiger Temperaturbereich	Manufacturer Fabricant Hersteller	REF Catalog Number Référence du catalogue Bestellnummer
Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten	Expiration Date Utiliser jusque Verwendbar bis	LOT Lot Number Code du lot Chargenbezeichnung

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