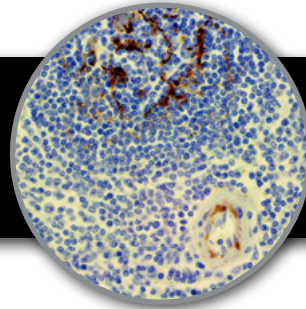


## C4d, RMab

Clone: EP272

Rabbit Monoclonal



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*Inset: IHC of C4d on a FFPE Spleen Tissue*

### Intended Use

For In Vitro Diagnostic Use.

This antibody is intended for use in Immunohistochemical 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.

<b>Antibody Type</b>	Rabbit Monoclonal	<b>Clone</b>	EP272
<b>Isotype</b>	IgG	<b>Reactivity</b>	Paraffin, Frozen
<b>Localization</b>	Cytoplasmic, Membranous	<b>Control</b>	Tonsil, Lymph Node, Spleen, Kidney, Kidney Transplant Rejection
<b>Species Reactivity</b>	Human		

### 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.

### Presentations

Catalog Num.	Antibody Type	Dilution	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	1:50 - 1:200	0.1 mL
BSB 2835	Concentrated	1:50 - 1:200	0.5 mL
BSB 2836	Concentrated	1:50 - 1:200	1.0 mL
BSB 2837	Control Slides	Not Applicable	5 slides

### Precautions

1. For professional users only. Ensure results are interpreted by a medical professional.
2. This product contains sodium azide (NaN<sub>3</sub>), a toxic chemical which may react with plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent sodium azide build-up.
3. Ensure proper handling procedures are used with reagent. Always wear proper laboratory equipment such as laboratory coat and gloves when handling reagents.
4. Unused solution should be disposed of according to local and federal regulations.
5. Do not ingest reagent. If reagent ingested, contact a poison control center immediately.
6. 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" (7).

### Storage

**Store at 2-8 °C.** 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 to ensure 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

1. Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positive 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 epitope retrieval 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 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. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
8. Wash slides with IHC wash buffer or DI water.
9. Continue IHC staining protocol.

## Recommended IHC Protocol

Step	ImmunoDetector AP/HRP	PolyDetector AP/HRP	PolyDetector Plus 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	Varies	Varies	Varies





## Product Limitations

Due to inherent variability present in immunohistochemical procedures (including fixation time of tissues, dilution factor of antibody, retrieval method utilized and incubation time), optimal performance should be established through the use of positive and negative controls. Results should be interpreted by a medical professional.

## References

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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

<b>EC</b> <b>REP</b>	EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands		Storage Temperature Limites de température Zulässiger Temperaturbereich		Manufacturer Fabricant Hersteller	<b>REF</b>	Catalog Number Référence du catalogue Bestellnummer
<b>IVD</b>	In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum		Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten		Expiration Date Utiliser jusque Verwendbar bis	<b>LOT</b>	Lot Number Code du lot Chargenbezeichnung

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