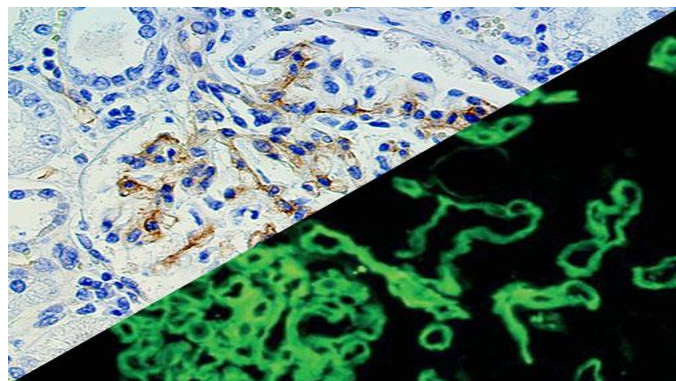


# C4d

**Clone:** EP272

Rabbit Monoclonal



*Inset: IHC of C4d on a FFPE Kidney Tissue; IF of C4d on a Frozen Kidney Rejection 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 Anti-C4d, 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>Species Reactivity</b>	Human, Rat, Mouse
<b>Control</b>	Tonsil, Lymph Node, Spleen, Kidney, Kidney Transplant Rejection		
<b>Application</b>	Kidney & Urothelial Cancer, Rejection & Autoimmunity		

## Presentation

Anti-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.

<b>Catalog No.</b>	<b>Presentation</b>	<b>Dilution</b>	<b>Volume</b>
BSB 2831	Predilute	Ready-to-Use	3.0 mL
BSB 2832	Predilute	Ready-to-Use	7.0 mL
BSB 2833	Predilute	Ready-to-Use	15.0 mL
BSB 2834	Concentrate	1:50 - 1:200	0.1 mL
BSB 2835	Concentrate	1:50 - 1:200	0.5 mL
BSB 2836	Concentrate	1:50 - 1:200	1.0 mL

## Control Slides Available

<b>Catalog No.</b>	<b>Quantity</b>
BSB-9045-CS	5 slides

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

## Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. This product contains <0.1% sodium azide (NaN<sub>3</sub>) as a preservative. Ensure proper handling procedures are used with this reagent.
3. Always wear personal protective equipment such as a laboratory coat, goggles, and gloves when handling reagents.
4. Dispose of unused solution with copious amounts 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).

## 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 on acetone-fixed frozen sections and acetone-fixed cell preparations.

#### IHC & IF Protocol

##### Preparation for Frozen Tissues Procedure

1. Embed the specimen in OCT inside the cryostat.
2. Cut sections at 5 microns.
3. Place the section on a positively charged glass slide.
4. Air dry for 30-60 minutes.
5. Fix in acetone 100% for 2-10 minutes.
6. Air dry for another 10 minutes.

##### Preparation for FFPE Tissues Procedure

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. For manual IF/IHC, perform antibody incubation at ambient temperature. For automated IF/IHC methods, perform antibody incubation according to instrument manufacturer's instructions.
8. Wash slides with ImmunoDNA washer or DI water.
9. Continue IF/IHC protocol. Wash slides between each step with ImmunoDNA washer solution.

#### Abbreviated Immunohistochemical 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 / Coverslip	Varies	Varies	Varies

#### Abbreviated AmpliDetector Plus FITC IF Protocol

Step	Incubation Time
Rinse slides in IF wash buffer	
Drain and wipe excess IF wash buffer off slide	
Peroxidase Blocker	5 min.
Apply Antibody	5 min.
Apply Mouse/Rabbit Link	5 min.
Apply HRP Label	5 min.
*Keep FITC reagents and slides in the dark*	
Apply AmpliDetector FITC solution	5 min.
Coverslip with IF mounting medium	

#### Mounting Protocol 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.

#### Mounting Protocol IF:

1. Bring FluoroMounter or FluoroMounter with DAPI to room temperature.
2. Rinse slides with distilled or deionized water.
3. Remove excess 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 °C in the dark.





#### 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 qualified medical professional.

#### References

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5. Nadasdy GM, et al. Hum Pathol. 2005 Nov; 36(11):1178-85.
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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.  
<https://www.cdc.gov/mmwr/pdf/other/su6101.pdf>

#### Symbol Key / Légende des symboles/Erläuterung der Symbole

<b>EC</b> <b>REP</b>	QAdvis EAR AB Ideon Science Park Scheelevägen 17 SE-223 70 Lund, Sweden	 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