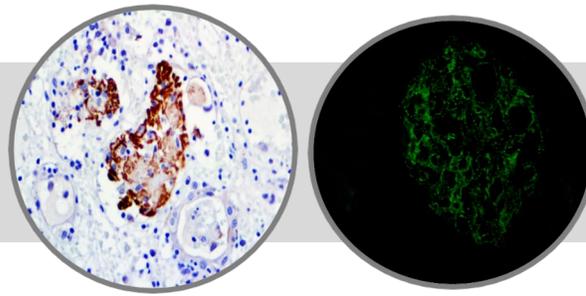


C3d

Clone: Polyclonal
Rabbit Polyclonal

**RUO**

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Inset: IHC and IF of C3d on a FFPE Rejected Kidney Transplant (IHC) and on a Frozen Lupus Erythematosus 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.

Immunogen

Purified full length native protein corresponding to the human C7 protein.

Summary and Explanation

Complement component 3, or C3, is a protein of the immune system that plays a central role in the complement system and contributes to innate immunity. Its activation is required for both classical and alternative complement activation pathways. C3d deposition in the renal transplant PTCs (peritubular capillaries) is indicative of AR (acute rejection) with subsequent high probability of graft loss.

Anti-C3d combined with anti-C4d can be utilized as a tool for diagnosis of AR and warrant prompt and aggressive anti-rejection treatment. C3d is also a helpful adjunct in the diagnosis of bullous pemphigoid (BP) and perhaps pemphigus vulgaris (PV), especially in the cases in which only formalin-fixed, paraffin embedded tissue is available for analysis.

Antibody Type	Rabbit Polyclonal	Clone	Polyclonal
Isotype	IgG	Reactivity	Paraffin, Frozen
Localization	Cytoplasmic, Membranous	Control	Rejected Kidney Transplant
Species Reactivity		Human	

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.

Presentation

C3d is a purified immunoglobulin fraction of rabbit antiserum that is 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 6387	Tinto Prediluted	Ready-to-Use*	3.0 mL
BSB 6388	Tinto Prediluted	Ready-to-Use*	7.0 mL
BSB 6389	Tinto Prediluted	Ready-to-Use*	15.0 mL
BSB 6390	Concentrated	1:100 / 1:500	0.1 mL
BSB 6391	Concentrated	1:100 / 1:500	0.5 mL
BSB 6392	Concentrated	1:100 / 1:500	1.0 mL

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

Control Slides Available

Catalog No.	Quantity
BSB 6393	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 (NaN₃) 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).

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 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% 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 and 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 °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 through the use of positive and negative controls. Results should be interpreted by a qualified medical professional.

References

1. Bickerstaff A, et al. Am J Pathology. 2008 Aug; 173(2):347-57.
2. Kuypers D, et al. Transplantation. 2003 Jul 15; 76(1):102-8.
3. Pfaltz K, et al. J Cutan Pathol. 2009 Oct 15.
4. Eggersten G, et al. APMIS. 2001 Dec; 109(12):825-34
5. 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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