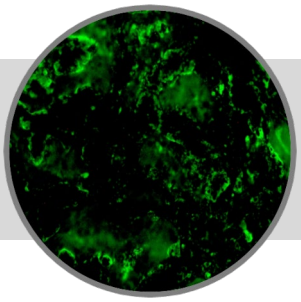
# C3c / FITC, RPab



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## Intended Use
For Research Use Only.

## Immunogen
Purified C3c protein isolated from normal human serum.

## Summary and Explanation
Complement component 3, often simply called C3, is a protein of the immune system. It plays a central role in the complement system and contributes to innate immunity.

C3 glomerulopathy was recently coined to describe renal biopsy appearances characterized by the presence of glomerular deposits composed predominantly of C3 in the absence of significant amounts of Ig. The presence of C3 in the absence of Ig suggests activation of complement by antibody-independent pathways, typically the alternative pathway, and many patients with this type of renal lesion have evidence of genetic or acquired alternative pathway dysregulation. C3 glomerulopathy has been further divided into dense deposit disease (DDD) and C3 glomerulonephritis (C3GN) based on electron microscopy (EM) appearances. The underlying genetic defect has been identified in some hereditary forms of C3GN such as CFHR5 nephropathy. Lupus nephritis is an inflammation of the kidneys caused by Systemic Lupus Erythematosus. Immunofluorescence reveals positively for IgG, IgA, IgM, C3, and C1q.

| | | | |
|---|---|---|---|
| **Antibody Type** | Rabbit Polyclonal | **Clone** | Polyclonal |
| **Isotype** | IgG | **Reactivity** | Paraffin, Frozen |
| **Localization** | Cytoplasmic, Membranous | **Control** | Placenta, Kidney, Fallopian Tube, Lupus Erythematosus |
| | **Species Reactivity** | Human | |

## 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
The C3c/FITC is a purified rabbit polyclonal antibody labeled with FITC diluted in a Tris Buffered Saline solution (pH 7.2) containing stabilizing proteins and preserved with sodium azide. It is provided in liquid form.

| *Catalog No.* | *Antibody Type* | *Dilution* | *Volume/Qty* |
|---|---|---|---|
| BSB-3002-3 | Tinto Predilute | Ready-to-Use | 3.0 mL |
| BSB-3002-7 | Tinto Predilute | Ready-to-Use | 7.0 mL |
| BSB-3002-15 | Tinto Predilute | Ready-to-Use | 15.0 mL |
| BSB-3002-05 | Concentrate | 1:25 - 1:100 | 0.5 mL |
| BSB-3002-1 | Concentrate | 1:25 - 1:100 | 1.0 mL |

## Control Slides Available

| *Catalog No.* | *Quantity* |
|---|---|
| BSB-3002-CS | 5 slides |

**Storage** Store at 2-8°C in the dark

## 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.

## Preparation of Working Solution
This FITC antibody is supplied as a concentrated solution and should be further diluted to a working concentration with appropriate antibody diluent. ImmunoDetector Protein Block/Antibody Diluent (BSB 0040 and BSB 0041) is suggested immediately prior to use.

## 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). 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.

## Preparation for Frozen Tissues Staining Procedure

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

## Preparation for FFPE Tissues Staining 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. 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 IF staining protocol.

## Abbreviated Protocol FITC Detection

| Step | Incubation Time |
|---|---|
| Rinse slides in IF wash buffer | 5 min |
| Drain and wipe excess IF wash buffer off slide | |
| Conduct remaining steps in the dark | |
| Apply Antibody | 30-60 min. |
| Rinse with 3 changes of IF wash buffer | 3x5 min. each |
| Coverslip with IF mounting medium | |

## Mounting Protocols

### 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 immunofluorescent 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

1. Sahu A, Lambris JD (Apr 2001). "Structure and biology of complement protein C3, a connecting link between innate and acquired immunity". Immunological Reviews. 2001; 180: 35–48.
2. Tamura T, et al. A case of recurrent proliferative glomerulonephritis with monoclonal IgG deposits or de novo C3 glomerulonephritis after kidney transplantation. Nephrology (Carlton). 2018 Jul;23 Suppl 2:76-80.
3. Medjeral-Thomas, et al. C3 Glomerulopathy: Clinicopathologic Features and Predictors of Outcome. Clin J Am Soc Nephrol 2014; 9: 46–53.
4. Benz RL. Immunoglobulin M nephropathy in a patient with systemic lupus. Am J Med Sci. 2011 Dec;342(6):530-2.
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<br>Limites de température<br>Zulässiger Temperaturbereich | Manufacturer<br>Fabricant<br>Hersteller | REF<br>Catalog Number<br>Référence du catalogue<br>Bestellnummer |
| Read Instructions for Use<br>Consulter les instructions d'utilisation<br>Gebrauchsanweisung beachten | Expiration Date<br>Utiliser jusque<br>Verwendbar bis | LOT<br>Lot Number<br>Code du lot<br>Chargenbezeichnung |

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