

Inset: IHC and IF of C1q on a FFPE Lupus Erythematosus (IHC) and a Frozen Lupus Erythematosus Tissue (IF)

# **Intended** Use

For In Vitro Diagnostic Use.

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

KLH conjugated synthetic peptide corresponding to the C-terminus region of human C1QA.

## **Summary and Explanation**

The complement component 1q (C1q) is a protein complex involved in the complement system, which is part of the innate immune system. C1q together with C1r and C1s form the C1 complex. Antibodies of the adaptive immune system can bind antigen, forming an antigen-antibody complex. When C1q binds antigenantibody complexes, the C1 complex becomes activated. Activation of the C1 complex initiates the classical complement pathway of the complement system.

C1q nephropathy is a rare glomerular disease with characteristic mesangial C1q deposition noted on IHC or IF microscopy. It is histologically defined and poorly understood. Light microscopic features are heterogeneous and comprise minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS), and proliferative glomerulonephritis. Clinical presentation is also diverse, and ranges from asymptomatic hematuria or proteinuria to frank nephritic or nephrotic syndrome in both children and adults. Hypertension and renal insufficiency at the time of diagnosis are common findings. 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		
lsotype	lgG	Reactivity	Paraffin, Frozen		
Localization	Cytoplasmic, Membranous	Control	Kidney, Cervix, Spleen, 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<sub>3</sub>) 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

C1q 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 3019	Tinto Prediluted	Ready-to-Use*	3.0 mL	
BSB 3020	Tinto Prediluted	Ready-to-Use*	7.0 mL	
BSB 3021	Tinto Prediluted	Ready-to-Use*	15.0 mL	
BSB 3022	Concentrated	1:50 / 1:100	0.1 mL	
BSB 3023	Concentrated	1:50 / 1:100	0.5 mL	
BSB 3024	Concentrated	1:50 / 1:100	1.0 mL	

\*Ready-to-Use, for IHC only

## **Control Slides Available**

Catalog No.	Quantity		
BSB 3025	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% 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 °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. Sellar GC, et al. (March 1991). "Characterization and organization of the genes encoding the A-, B- and C-chains of human complement subcomponent C1q. The complete derived amino acid sequence of human C1q". Biochem. J. 1991; 274 (2): 481–90.

2. Markowitz, G. S. et al. C1q nephropathy: a variant of focal segmental

glomerulosclerosis. Kidney International, 2003; 64, 232–1240.

3. Devasahayam, J. et al., C1q Nephropathy: The Unique Underrecognized Pathology Entity. Analytical Cellular Pathology. 2015; https://www.hindawi.com/journals/acp/2015/490413/ 4. Alenka Vizjak, et al. Pathology, Clinical Presentations, and Outcomes of C1q Nephropathy. J Am Soc Nephrol. 2008; 19 (11): 2237-2244.

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

	-arc	Storage Temperature Limites de température		Manufacturer Fabricant	REF	Catalog Number Référence du catalogue
	20	Zulässiger Temperaturbereich		Hersteller		Bestellnummer
IVD In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum	[]i	Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten	$\sum$	Expiration Date Utiliser jusque Verwendbar bis	LOT	Lot Number Code du lot Chargenbezeichnung

