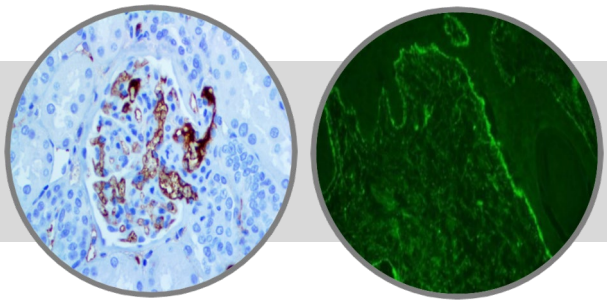
PI 3085, Rev. A DCN: 3771

IgM

Clone: Polyclonal

Rabbit Polyclonal



IVD

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Inset: IHC and IF of IgM on a FFPE Kidney Tissue (IHC) and on 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

Purified human IgM heavy chain.

Summary and Explanation

IgM forms polymers where multiple immunoglobulins are covalently linked together with disulfide bonds, normally as a pentamer or occasionally as a hexamer. It has a large molecular mass of approximately 900 kDa (in its pentamer form). In germline cells, the gene segment encoding the constant region of the heavy chain is positioned first among other constant region gene segments. For this reason, IgM is the first immunoglobulin expressed by mature B-cells.

IgM antibody reacts with surface immunoglobulin IgM mu chains. IgM is one of the predominant surface immunoglobulins on B-lymphocytes, and is useful when identifying Leukemias, Plasmacytomas, and B-cell lineage derived Hodgkin's Lymphomas. Due to the restricted expression of heavy and light chains in these diseases, demonstration of B-cell Lymphomas is possible with clonal gene-rearrangement studies. Lupus nephritis is an inflammation of the kidneys caused by Systemic Lupus Erythematosus. Immunofluorescence reveals positively for IgG, IgA, IgM, C3, and C1q. Clinically, hematuria and proteinuria are present, with or without nephrotic syndromes. Immunoglobulin M (IgM) nephropathy is an uncommon glomerular disease characterized by IgM deposits in the mesangium.

Antibody Type	Rabbit Polyclonal	**Clone**	Polyclonal
Isotype	IgG	**Reactivity**	Paraffin, Frozen
Localization	Cytoplasmic	**Control**	Tonsil, Lymph Node, Spleen, Kidney, Colon
	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

IgM 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 3080	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 3081	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 3082	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 3083	Concentrated	1:100 / 1:40	0.1 mL
BSB 3084	Concentrated	1:100 / 1:40	0.5 mL
BSB 3085	Concentrated	1:100 / 1:40	1.0 mL

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

Control Slides Available

Catalog No.	*Quantity*
BSB 3687	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_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).

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 ^{0}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. Arnold A, et al. New Eng J Med. 1983;309:1593-1599
2. Taylor CR, et al. Ibid. pp179-202
3. Hertel BF, et al. New Eng J Med. 1980;302:1293-1297
4. Warnake R, et al. Masson Publishing USA. 1981;pp203-221
5. Curran RC, Gregory J, J Clin Pathol. 1978;31:974
6. Benz RL. Immunoglobulin M nephropathy in a patient with systemic lupus. Am J Med Sci. 2011 Dec;342(6):530-2.
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

	Storage Temperature Limites de température Zulässiger Temperaturbereich	Manufacturer Fabricant Hersteller	REF Catalog Number Référence du catalogue Bestellnummer
IVD 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	LOT Lot Number Code du lot Chargenbezeichnung

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