# **Mycobacterium tuberculosis**

**Clone: Polyclonal Rabbit Polyclonal** 



Inset: IHC of Mycobacterium tuberculosis on a FFPE Infected Lung Tissue

# **Intended Use**

For In Vitro Diagnostic Use.

This antibody is intended for use in Immunohistochemical applications on formalinfixed paraffin-embedded tissues (FFPE), frozen tissue sections and cell preparations. Interpretation of results should be performed by a qualified medical professional.

#### **Immunogen**

Purified PPD.

# **Summary and Explanation**

Mycobacterium tuberculosis is a pathogenic bacterial species of the Mycobacteriaceae family and the causative agent of most cases of tuberculosis. M. tuberculosis has an unusual, waxy coating on its cell surface (primarily due to the presence of mycolic acid), which makes the cells impervious to Gram staining; M. tuberculosis can appear Gram negative and Gram positive in clinical settings. The Ziehl-Neelsen stain, or acidfast stain, is used instead. M. tuberculosis is highly aerobic and requires high levels of oxygen. Humans are the only known reservoirs of M. tuberculosis. When in the lungs, M. tuberculosis is taken up by alveolar macrophages, but they are unable to digest and eradicate the bacterium. Its cell wall prevents the fusion of the phagosome with lysosome, which contains a host of antimycobacterial factors. Antibiotic resistant strains of mycobacterium tuberculosis have developed resistance to more than one TB drug, due to mutations in their genes.

M. tuberculosis is characterized by caseating granulomas containing Langhans giant cells, which have a "horseshoe" pattern of nuclei. Cells are often seen wrapped together, due to the presence of fatty acids in the cell wall that stick together. This appearance is referred to as chording, like strands of chord that make up a rope. The clinical and histological criteria used to diagnose lymphadenitis caused by Mycobacterium tuberculosis complex organisms have poor specificity. Acid-fast staining and culture have low sensitivity and specificity. The diagnosis of tuberculosis by immunohistochemistry can be used to detect the mycobacterial antigen on formalin-fixed tissue biopsies and it's consider fast, sensitive, and a highly specific method for establishing the etiological diagnosis of tuberculosis in histologic specimens. Mycobacterium tuberculosis RPab has shown crossreactivity with aspergillus fumigatus and gram negative bacteria.

Antibody Type	Rabbit Polyclonal	Clone	C <b>lone</b> Polyclonal		
Isotype	lgG	Reactivity	Paraffin, Frozen		
Localization	Cell Wall	Control	Infected Tissue		
Species Reactivity		Human			

## **Presentation**

Mycobacterium tuberculosis 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	Dilution	Volume/Qty	
BSB 2992	Tinto Prediluted	Ready-to-Use	3.0 mL	
BSB 2993	Tinto Prediluted	Ready-to-Use	7.0 mL	
BSB 2994	Tinto Prediluted	Ready-to-Use	15.0 mL	
BSB 2995	Concentrated	1:250 - 1: 1000	0.1 mL	
BSB 2996	Concentrated	1:250 - 1: 1000	0.5 mL	
BSB 2997	Concentrated	1:250 - 1: 1000	1.0 mL	

#### **Control Slides Available**

Catalog No.	Quantity		
BSB 2998	5 slides		

## **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).

# Storage Store at 2-8°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.

## PI 2997, Rev. E DCN: 3201

# **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**

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

## **Abbreviated Immunohistochemical Protocol**

Step	ImmunoDetector PolyDetector AP/HRP AP/HRI		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	

# **Mounting Protocols**

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 Pl0174 or Pl0097.

#### **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

- 1. Martínez, A., et al. Sliding Motility in Mycobacteria. J Bacteriol. 1999; 181 (23): 7331–7338.
- 2. Fu, L. M.; Fu-Liu, C. S. Is Mycobacterium tuberculosis a closer relative to Grampositive or Gram-negative bacterial pathogens? Tuberculosis (Edinburgh, Scotland, 2002; 82 (2-3): 85–90.
- 3. Keane J, et al. Infection by Mycobacterium tuberculosis promotes human alveolar macrophage apoptosis. Infect. Immun.1997: 65 (1): 298–304.
- 4. Mustafa T, et al. Immunohistochemistry using a Mycobacterium tuberculosis complex specific antibody for improved diagnosis of tuberculous lymphadenitis. Mod Pathol. 2006; 19(12):1606-14.
- 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.

## Not for Sale in the USA

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

EC R	EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands	270 arc	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	$\bigcap_{\mathbf{i}}$	Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten	$\subseteq$	Expiration Date Utiliser jusque Verwendbar bis	LOT	Lot Number Code du lot Chargenbezeichnung

