**Mycobacterium tuberculosis**

**Clone:** Polyclonal
**Rabbit Polyclonal**

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**Intended Use**

For In Vitro Diagnostic Use.

This antibody is intended for use in immunohistochemical 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.

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**Immunogen**

Purified PPD.

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**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 acid-fast 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 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.

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**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.

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**Antibody Type**

Rabbit Polyclonal

**Clone**

Polyclonal

**Isotype**

IgG

**Reactivity**

Paraffin, Frozen

**Localization**

Cell Wall

**Control**

Infected Tissue

**Species Reactivity**

Human

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**Precautions**

1. For professional users only. Ensure results are interpreted by a medical professional.
2. This product contains sodium azide (NaN₃), a toxic chemical which may react with plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent sodium azide build-up.
3. Ensure proper handling procedures are used with reagent. Always wear proper laboratory equipment such as laboratory coat and gloves when handling reagents.
4. Unused solution should be disposed of according to local and federal regulations.
5. Do not ingest reagent. If reagent ingested, contact a poison control center immediately.

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**Storage**

Store at 2-8 °C. 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.

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**Parafin sections**

The antibody can be used on formalin-fixed paraffin-embedded (FFPE) tissue sections. Ensure tissue undergoes appropriate fixation to ensure 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.

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**Presentations**

<table>
<thead>
<tr>
<th>Catalog Num.</th>
<th>Antibody Type</th>
<th>Dilution</th>
<th>Volume/Qty</th>
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</thead>
<tbody>
<tr>
<td>BSB 2992</td>
<td>Tinto Prediluted</td>
<td>Ready-to-Use</td>
<td>3.0 mL</td>
</tr>
<tr>
<td>BSB 2993</td>
<td>Tinto Prediluted</td>
<td>Ready-to-Use</td>
<td>7.0 mL</td>
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<tr>
<td>BSB 2994</td>
<td>Tinto Prediluted</td>
<td>Ready-to-Use</td>
<td>15.0 mL</td>
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<tr>
<td>BSB 2995</td>
<td>Concentrated</td>
<td>1:100 - 1:500</td>
<td>0.1 mL</td>
</tr>
<tr>
<td>BSB 2996</td>
<td>Concentrated</td>
<td>1:100 - 1:500</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>BSB 2997</td>
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<td>1:100 - 1:500</td>
<td>1.0 mL</td>
</tr>
<tr>
<td>BSB 2998</td>
<td>Control Slides</td>
<td>Not Applicable</td>
<td>5 slides</td>
</tr>
</tbody>
</table>
**Staining Procedure**

1. Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positive 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 epitope retrieval 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 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 IHC wash buffer or DI water.
9. Continue IHC staining protocol.

**Recommended IHC Protocol**

<table>
<thead>
<tr>
<th>Step</th>
<th>ImmunoDetector AP/HRP</th>
<th>PolyDetector AP/HRP</th>
<th>PolyDetector Plus HRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroxidase/AP Blocker</td>
<td>5 min.</td>
<td>5 min.</td>
<td>5 min.</td>
</tr>
<tr>
<td>Primary Antibody</td>
<td>30-60 min.</td>
<td>30-60 min.</td>
<td>30-60 min.</td>
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<tr>
<td>1st Step Detection</td>
<td>10 min.</td>
<td>30-45 min.</td>
<td>15 min.</td>
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<tr>
<td>2nd Step Detection</td>
<td>10 min.</td>
<td>Not Applicable</td>
<td>15 min.</td>
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<tr>
<td>Substrate-Chromogen</td>
<td>5-10 min.</td>
<td>5-10 min.</td>
<td>5-10 min.</td>
</tr>
<tr>
<td>Counterstain</td>
<td>Varies</td>
<td>Varies</td>
<td>Varies</td>
</tr>
</tbody>
</table>

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

**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 medical professional.

**References**