**Intended Use**

For Research Use Only.

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.

**Immunogen**

Purified human Factor VIII.

**Summary and Explanation**

Factor VIII (F VIII) is an essential clotting factor. The lack of normal F VIII causes Hemophilia A, an inherited bleeding disorder. FVIII is a glycoprotein procofactor synthesized and released into the bloodstream by the liver. In the circulating blood, it is mainly bound to von Willebrand factor (vWF, also known as Factor VIII-related antigen) to form a stable complex. Upon activation by thrombin or Factor Xa, it dissociates from the complex to interact with Factor IXa, the coagulation cascade. It is a cofactor to Factor IXa in the activation of Factor X, which, in turn, with its cofactor Factor Va, activates more thrombin. Thrombin cleaves fibrinogen into fibrin which polymerizes and crosslinks (using Factor XIII) into a blood clot.

This antibody reacts with endothelial cells in normal, reactive, and neoplastic blood cells. F VIII antibody has helped to establish the endothelial nature of some lesions of disputed histogenesis, e.g., Kaposi’s Sarcoma and Cardiac Myxoma. Not all endothelial cells synthesize (or store) this molecule; therefore, it should not be surprising that not all tumors of endothelial differentiation (benign or malignant) react with this antigen.

**Presentations**

<table>
<thead>
<tr>
<th>Catalog Num.</th>
<th>Antibody Type</th>
<th>Dilution</th>
<th>Volume/Qty</th>
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<tbody>
<tr>
<td>BSB 5498</td>
<td>Tinto Prediluted</td>
<td>Ready-to-Use</td>
<td>3.0 mL</td>
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<tr>
<td>BSB 5499</td>
<td>Tinto Prediluted</td>
<td>Ready-to-Use</td>
<td>7.0 mL</td>
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<tr>
<td>BSB 5500</td>
<td>Tinto Prediluted</td>
<td>Ready-to-Use</td>
<td>15.0 mL</td>
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<tr>
<td>BSB 5501</td>
<td>Concentrated</td>
<td>1:50 - 1:200</td>
<td>0.1 mL</td>
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<tr>
<td>BSB 5502</td>
<td>Concentrated</td>
<td>1:50 - 1:200</td>
<td>0.5 mL</td>
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<tr>
<td>BSB 5503</td>
<td>Concentrated</td>
<td>1:50 - 1:200</td>
<td>1.0 mL</td>
</tr>
<tr>
<td>BSB 5504</td>
<td>Control Slides</td>
<td>Not Applicable</td>
<td>5 slides</td>
</tr>
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</table>

**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.

**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.

**Specimen Preparation**

**Paraffin 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.
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>
</tr>
<tr>
<td>1st Step Detection</td>
<td>10 min.</td>
<td>30-45 min.</td>
<td>15 min.</td>
</tr>
<tr>
<td>2nd Step Detection</td>
<td>10 min.</td>
<td>Not Applicable</td>
<td>15 min.</td>
</tr>
<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>

Performance Characteristics

Normal Tissues

- Positive (+)
  - megakaryocytes
  - platelets
  - endothelial cells lining the lumen of capillaries, lymphatic vessels, arteries and veins
  - endothelial cells in the sinusoids of the liver and spleen
  - endothelial cells in glomeruli

Abnormal Tissues

- Positive (+)
  - epithelioid hemangoendothelioma of the liver, the epithelioid, dendritic or mediate cells of the malignant vascular tissue showed strong expression of von Willebrand factor (99% of 137 patients)

- Negative (-)
  - von Willebrand factor is seldom expressed in poorly differentiated vascular tumors

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