**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.

* The CD163 antibody, clone EP324, has been manufactured using Epitomics RabMab® technology covered under Patent No.'s 5,675,063 and 7,402,409.

**Immunogen**
A synthetic peptide corresponding to residues of human CD163 protein.

**Summary and Explanation**
CD163 is a protein that in humans is encoded by the CD163 gene. CD163 is the high affinity scavenger receptor for the hemoglobin-haptoglobin complex and in the absence of haptoglobin - with lower affinity - for hemoglobin alone. CD163 is expressed exclusively on the cell surface of human monocytes and macrophages that evolve predominantly in the late phase of inflammation, and is, therefore, very useful for macrophage-phenotyping. A soluble form of the receptor exists in plasma, commonly named sCD163, which is upregulated in a large range of inflammatory diseases including liver cirrhosis, type 2 diabetes, atherosclerosis, macrophage activation syndrome, Gaucher's disease, sepsis, HIV infection, rheumatoid arthritis and Hodgkin Lymphoma.

CD163 positivity by IHC can be seen in histiocytes, gut, Kupffer cells, a few alveolar macrophages, the main population of macrophages in the placenta, and in varying degrees in macrophages in inflamed tissue including tumor tissue, depending on the inflammatory stage. Red-pulp, not white-pulp, macrophages in the spleen and cortical macrophages of the thymus are also positive for this marker. CD163 positivity may also be seen in tumor tissue, depending on the inflammatory stage. Red-pulp, not white-pulp, macrophages in the spleen and cortical macrophages of the thymus are also positive for this marker. CD163 has been found to be helpful in distinguishing synovial macrophages from synovial intimal fibroblasts in the setting of rheumatoid arthritis, with superior specificity for macrophages than CD68, which does not discriminate between these cell types. It also has been confirmed in previous reports of having a prognostic role of tumor-infiltrating macrophages in classical Hodgkin's Lymphoma. Increased levels of CD163 have been detected in patients with microbial infections and myelomonocytic leukemias and studies have confirmed the fact that CD163 expression is limited to leukemias with monocytic differentiation. Another recent study showed that all 5 cases of synovial-type giant cell tumors of the parietal pericardium displayed CD163 positivity by IHC.

**Presentation**
C4d is a rabbit monoclonal antibody derived from cell culture supernatant that is concentrated, dialyzed, filter sterilized and diluted in buffer pH 7.5, containing BSA and sodium azide as a preservative.

**Presentations**

<table>
<thead>
<tr>
<th>Catalog Num.</th>
<th>Antibody Type</th>
<th>Dilution</th>
<th>Volume/Qty</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSB 3272</td>
<td>Tinto Prediluted</td>
<td>Ready-to-Use</td>
<td>3.0 mL</td>
</tr>
<tr>
<td>BSB 3273</td>
<td>Tinto Prediluted</td>
<td>Ready-to-Use</td>
<td>7.0 mL</td>
</tr>
<tr>
<td>BSB 3274</td>
<td>Tinto Prediluted</td>
<td>Ready-to-Use</td>
<td>15.0 mL</td>
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<tr>
<td>BSB 3275</td>
<td>Concentrated</td>
<td>1:50 - 1:200</td>
<td>0.1 mL</td>
</tr>
<tr>
<td>BSB 3276</td>
<td>Concentrated</td>
<td>1:50 - 1:200</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>BSB 3277</td>
<td>Concentrated</td>
<td>1:50 - 1:200</td>
<td>1.0 mL</td>
</tr>
<tr>
<td>BSB 3278</td>
<td>Control Slides</td>
<td>Not Applicable</td>
<td>5 slides</td>
</tr>
</tbody>
</table>

**Precautions**
1. For professional users only. Ensure results are interpreted by a medical professional.
2. This product contains sodium azide (Na3N), 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.
6. 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" (5).

**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>

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

- **IVD** In Vitro Diagnostic Medical Device
- **Manufacturer** Fabricant / Hersteller
- **Lot Number** Code du lot
- **Storage Temperature** Limites de température
- **Expiration Date** Utiliser jusque
- **Read Instructions for Use** Consulter les instructions d’utilisation
- **Symbol Key** Référence du catalogue
- **Catalog Number** Bestellnummern

**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**