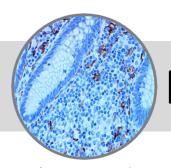
CD163, RMab

Clone: EP324
Rabbit Monoclonal







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Inset: IHC of CD163 on a FFPE Colon Tissue

Intended Use

For Research Use Only.

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.

* 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 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 spinal column were positive for CD163.

| Antibody Type | Rabbit Monoclonal | Clone | EP324 | |
|---------------|----------------------------|------------|--|--|
| Isotype | lgG | Reactivity | y Paraffin, Frozen | |
| Localization | Cytoplasmic, Membranous | Control | Placenta, Tonsil, Lymph Node, Inflamed Tissue, H. Pylori Infected Tissues | |
| | Species Reactivity | Human | | |

Presentation

CD163 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.

| Catalog No. | Antibody Type | Dilution | Volume/Qty |
|-------------|------------------|--------------|------------|
| BSB 3272 | Tinto Prediluted | Ready-to-Use | 3.0 mL |
| BSB 3273 | Tinto Prediluted | Ready-to-Use | 7.0 mL |
| BSB 3274 | Tinto Prediluted | Ready-to-Use | 15.0 mL |
| BSB 3275 | Concentrated | 1:50 - 1:200 | 0.1 mL |
| BSB 3276 | Concentrated | 1:50 - 1:200 | 0.5 mL |
| BSB 3277 | Concentrated | 1:50 - 1:200 | 1.0 mL |

Control Slides Available

| Catalog No. | Quantity | | |
|-------------|----------|--|--|
| BSB 3278 | 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.
- $5.\ Do\ not\ ingest\ reagent.\ If\ reagent\ is\ ingested,\ seek\ medical\ advice\ immediately.$
- $6. \ A void \ contact \ with \ eyes. \ \ If \ contact \ occurs, \ flush \ with \ large \ quantities \ of \ water.$
- 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).

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 3277_b, Rev. B DCN: 3396_b

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 AP/HRP | PolyDetector AP/HRP | 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. Schaer DJ., et al. CD163 is the macrophage scavenger receptor for native and chemically modified hemoglobins in the absence of haptoglobin. Blood. 2006 Jan 1;107(1):373-80.
- 2. Lau SK, et al. CD163: a specific marker of macrophages in paraffin-embedded tissues. Am. J. Clin. Path. 2004; 122 (5): 794–801.
- 3. Jones K, et al. Serum CD163 and TARC as disease response biomarkers in classical Hodgkin lymphoma. Clin. Cancer Res. 2013; 19 (3): 731–42.
- 4. Klein JL, et al. CD163 immunohistochemistry is superior to CD68 in predicting outcome in classical Hodgkin lymphoma. Am J Clin Pathol. 2014 Mar; 141 (3): 381-7.
- 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.

Symbol Key / Légende des symboles/Erläuterung der Symbole

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|--------------|--|--------|--|-----|---|
| | Storage Temperature | | Manufacturer | | Catalog Number |
| Varc. | Limites de température | | Fabricant | REF | Référence du catalogue |
| 2.0 | Zulässiger Temperaturbereich | | Hersteller | | Bestellnummer |
| []i | Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten | \leq | Expiration Date Utiliser jusque Verwendbar bis | LOT | Lot Number Code du lot Chargenbezeichnung |



