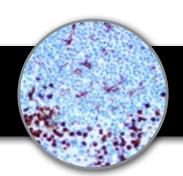
CD11b, RMab Clone: EP45 Rabbit Monoclonal

C€ IVD





Inset: IHC of CD11b on a FFPE Spleen Tissue

# **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 CD11b antibody, clone EP45, has been manufactured using Epitomics RabMab® technology covered under Patent No.'s 5,675,063 and 7,402,409.

### **Immunogen**

Synthetic peptide corresponding to residues in human CD11b protein.

# **Summary and Explanation**

Integrin alpha M (ITGAM) is one protein subunit that forms the heterodimeric integerin alpha-M beta-2 molecule, also known as macrophage-1 antigen (Mac-1) or complement receptor 3 (CR3). ITGAM is also known as CR3A, and cluster of differentiation molecule 11B (CD11b). The  $\alpha$ M $\beta$ 2 molecule is expressed on the surface of many leukocytes involved in the innate immune system, including monocytes, granulocytes, macrophages, and natural killer cells. It mediates inflammation by regulating luekocyte adhesion and migration and has been implicated in several immune processes such as phagocytosis, cell-mediated cytoxicity, chemotaxis and cellular activation. The ITGAM subunit of  $\alpha$ M $\beta$ 2 is directly involved in causing the adhesion and spreading of cells but cannot mediate cellular migration without the presence of the Beta-2 (CD18) subunit.

CD11b has been used as a common myeloid marker and is expressed in about 50% of acute myeloid leukemia (AML). In combination with CD117, CD11b is helpful in differentiating acute promyelocytic leukemia (CD11b negative) from recovering benign myeloid proliferation (CD11b positive, CD117 negative).

Antibody Type	Rabbit Monoclonal	Clone	EP45
Isotype	IgG	Reactivity	Paraffin, Frozen
Localization	Cytoplasmic	Control	Spleen, Leukemia
Species Reactivity		Human, Predicted: Mouse	

## **Presentation**

CD11b 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**

Catalog Num.	Antibody Type	Dilution	Volume/Qty
BSB 6436	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 6437	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 6438	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 6439	Concentrated	1:100 - 1:400	0.1 mL
BSB 6440	Concentrated	1:100 - 1:400	0.5 mL
BSB 6441	Concentrated	1:100 - 1:400	1.0 mL
BSB 6442	Control Slides	Not Applicable	5 slides

### **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.
- **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" (7).

### 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**

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	Varies	Varies	Varies

### **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

- 1. Solovjov D, et al. J Biol Chem. 280(2):1336-45
- 2. Larson R, et al. Immunol Rev. 114:181-217
- 3. Arnaout M, et al. J clin Invest. 72(1):171-9
- 4. Zhang T, et al. Mod Pathol. 2000; 13:954-61
- 5. Venditti A, et al. Blood. 1997; 89:621-9
- 6. Wakselman S, et al. J Neurosci. 2008; 28:8138-43
- 7. 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





