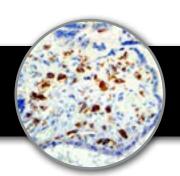
CD14, RMab Clone: EP128 Rabbit Monoclonal

C€ IVD





Inset: IHC of CD14 on a FFPE Placenta 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 CD14 antibody, clone EP128, 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 of human CD14 protein.

Summary and Explanation

CD14 is a component of the innate immune system. CD14 exists in two forms: mCD14 (anchored by a glycosylphosphatidylinositol tail) or sCD14 (soluble). CD14 acts as a co-receptor (along with the Toll-like receptor TLR 4 and MD-2) for the detection of bacterial lipopolysaccharide (LPS). CD14 can only bind LPS in the presence of lipopolysaccharide-binding protein (LBP). Although LPS is considered it's main ligand CD14 also recognizes other pathogen associated molecular patterns.

CD14 is expressed mainly by macrophages and (at 10 times lesser extent) by neutrophil granulocytes. A soluble form sCD14 is secreted by the liver and monocytes and is sufficient in low concentrations to confer LPS-responsiveness to cells which otherwise do not express CD14. sCD14 is also present in human milk where it is believed to regulate microbial growth in the infant gut. Increased sCD14 levels are associated with inflammatory infectious diseases and high mortality in gram-negative shock. CD14 also appears to be involved in clearance of gram-negative bacteria via its high affinity binding to LPS-LPB complexes.

Antibody Type	Rabbit Monoclonal	Clone	EP128
Isotype	IgG	Reactivity	Paraffin, Frozen
Localization	Cytoplasmic, Membranous	Control	Placenta, Tonsil, Spleen, Diffuse Large B-cell Lymphoma
Species Reactivity		Human	

Presentation

CD14 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 6457	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 6458	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 6459	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 6460	Concentrated	1:25 - 1:100	0.1 mL
BSB 6461	Concentrated	1:25 - 1:100	0.5 mL
BSB 6462	Concentrated	1:25 - 1:100	1.0 mL
BSB 6463	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" (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

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. Haziot A et al. J Immunol 1993; 150(12):5556-5565
- 2. Kitchens RL. Chem. Immunol. 2000;74: 61-82
- 3. Pugin J et al. Proc Natl Acad Sci USA 1993; 90(7):2744-2748
- 4. Wright SD et al. Science 1990; 249:1431-1433
- 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





