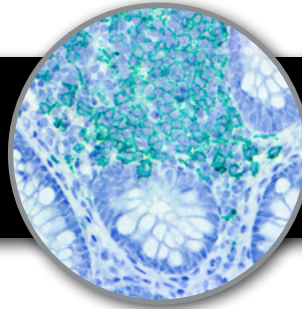


**CD3 Epsilon, RMab**  
 Clone: RBT-CD3e  
 Rabbit Monoclonal



*Inset: IHC of CD3 Epsilon on a FFPE Colon 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.

**Immunogen**

Synthetic peptide corresponding to residues of the epsilon chain of the human CD3 protein.

**Summary and Explanation**

The CD3 antigen is a protein complex composed of three distinct chains (CD3 $\gamma$ , CD3 $\delta$  and CD3 $\epsilon$ ) that associate with T-cell receptors and the  $\zeta$ -chain to generate an activation signal in T-lymphocytes. The TCR,  $\zeta$ -chain and CD3 molecules together comprise the TCR complex. The CD3 $\gamma$ , CD3 $\delta$ , and CD3 $\epsilon$  chains are highly-related cell surface proteins of the immunoglobulin superfamily containing a single extracellular immunoglobulin domain. The intracellular tails of the CD3 molecules contain a single conserved motif known as an immunoreceptor tyrosine-based activation motif (or ITAM for short), which is essential for the signaling capacity of the TCR. Phosphorylation of the ITAM on CD3 renders the CD3 chain capable of binding the enzyme ZAP70 (zeta-associated protein), a kinase important in the signaling cascade of the T-cell.

The epsilon polypeptide plays an essential role in T-cell development. Defects in this gene cause immunodeficiency and has also been linked to a susceptibility to type I diabetes in women. CD3 has been considered the best all-around T-cell marker. This antibody reacts with an antigen present in early thymocytes. The positive staining of this marker may represent a sign of early commitment to the T-cell lineage.

<b>Antibody Type</b>	Rabbit Monoclonal	<b>Clone</b>	RBT-CD3e
<b>Isotype</b>	IgG	<b>Reactivity</b>	Paraffin, Frozen
<b>Localization</b>	Membranous	<b>Control</b>	Tonsil, Lymph Node
<b>Species Reactivity</b>	Human		

**Presentation**

CD3e 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 5141	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 5142	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 5143	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 5144	Concentrated	1:100 - 1:500	0.1 mL
BSB 5145	Concentrated	1:100 - 1:500	0.5 mL
BSB 5146	Concentrated	1:100 - 1:500	1.0 mL
BSB 5147	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 (NaN<sub>3</sub>), 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. Denning SM, et al. Oxford Univ Press. 1987;144-147
2. Beverley PCL, et al. European J of Immunolgy. 11:329-334
3. Clevers H, et al. European J of Immunolgy. 1988;18:705-710
4. Meuer SC, et al. Immunology Today. 1989;10:255-228
5. Campana D, et al. J of Immunology. 1987;138:648-665
6. Abbas AK, Lichtman, Cellular and Molecular Immunology (5th Ed.) 2003
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

	EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands		Storage Temperature Limites de température Zulässiger Temperaturbereich		Manufacturer Fabricant Hersteller		Catalog Number Référence du catalogue Bestellnummer
	In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum		Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten		Expiration Date Utiliser jusque Verwendbar bis		Lot Number Code du lot Chargenbezeichnung

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