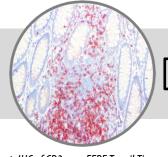
CD3, RMab Clone: RBT-CD3 Rabbit Monoclonal



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Inset: IHC of CD3 on a FFPE Tonsil Tissue

## **Intended** Use

For In Vitro Diagnostic Use.

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.

### Immunogen

Synthetic peptide corresponding to residues in the cytoplasmic domain 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.

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.

Antibody Type	Rabbit Monoclonal	Clone	RBT-CD3		
lsotype	lgG	Reactivity	Paraffin, Frozen		
Localization	Membranous	Control	Tonsil, Lymph Node, Liver, Testis, Kidney, Colon, Spleen, Thymus, Lymphoblastic Lymphoma		
	Species Reactivity	Human			

### Presentation

CD3 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 6422	Tinto Prediluted	Ready-to-Use	3.0 mL	
BSB 6423	Tinto Prediluted	Ready-to-Use	7.0 mL	
BSB 6424	Tinto Prediluted	Ready-to-Use	15.0 mL	
BSB 6425	Concentrated	1:50 - 1:200	0.1 mL	
BSB 6426	Concentrated	1:50 - 1:200	0.5 mL	
BSB 6427	Concentrated	1:50 - 1:200	1.0 mL	

### **Control Slides Available**

Catalog No.	Quantity		
BSB 6428	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<sub>3</sub>) 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. Avoid 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.

# **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.

4. 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.

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 PI0174 or PI0097.

# **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. 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 Immunolgy. 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

EC REP	EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands	200 arc	Storage Temperature Limites de température Zulässiger Temperaturbereich		Manufacturer Fabricant Hersteller	REF	Catalog Number Référence du catalogue Bestellnummer
IVD Di	In Vitro Diagnostic Medical Device ispositif médical de diagnostic in vitro In-Vitro-Diagnostikum	[]i	Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten	$\sum$	Expiration Date Utiliser jusque Verwendbar bis	LOT	Lot Number Code du lot Chargenbezeichnung



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