



CD3

IHC of CD3 on an FFPE Tonsil Tissue

Description The CD3 antigen is a protein complex composed of three distinct chains (CD3 γ , CD3 δ and CD3 ϵ) that associate with T-cell receptors and the ζ -chain to generate an activation signal in T-lymphocytes. The TCR, ζ -chain and CD3 molecules together comprise the TCR complex. The CD3 γ , CD3 δ , and CD3 ϵ 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
Isotype	IgG	Reactivity	Paraffin, Frozen
Localization	Membranous	Control	Tonsil, Lymph Node
Storage	Store at 2°-8°C	Stability	2 years

For long-term storage of the concentrated antibody, it is recommended that aliquots of the antibody be frozen at -20°C in glycerol 50% (frost-free freezers are not recommended). Repeated freezing and thawing must be avoided. Dilute using an antibody diluent such as ImmunoDetector Protein Block/Antibody Diluent (BSB 0040 and BSB 0041) or ImmunoDNA Background Blocker (BSB 0103-BSB 0107).

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.

Availability	Catalog No.	Antibody Type	Dilution	Volume/QTY
	BSB 5141	Prediluted	Ready-To-Use	3.0 ml
	BSB 5142	Prediluted	Ready-To-Use	7.0 ml
	BSB 5143	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		5

Note: For concentrated antibodies, please centrifuge prior to use to ensure recovery of all product.

- References**
- Denning SM, et al. *Oxford Univ Press*. 1987;144-147
 - Beverley PCL, et al. *European J of Immunology*. 11:329-334
 - Clevers H, et al. *European J of Immunology*. 1988;18:705-710
 - Meuer SC, et al. *Immunology Today*. 1989;10:255-228
 - Campana D, et al. *J of Immunology*. 1987;138:648-665
 - Abbas AK, Lichtman, *Cellular and Molecular Immunology (5th Ed.)* 2003

Protocol Suggested protocol on reverse

Recommended Immunohistochemical Protocol

- Pretreatment**
1. Cut and mount 3-4 micron formalin-fixed paraffin-embedded tissues on positive charged slides.
 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. **Electric Pressure Cooker**
Place standoff rack at base of pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high, and incubate for 15 minutes. Open and immediately transfer slides to room temperature.
 - b. **Water Bath Method**
Place tissues/slides in a pre-warmed staining dish or coplin jar containing the **ImmunoDNA Retriever with Citrate** or **EDTA** in a water bath set 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. Wash slides with IHC wash buffer or DI water.
 8. Continue IHC staining protocol.

Immunohistochemical Protocol

Step	ImmunoDetector (AP or HRP)	PolyDetector (AP or HRP)
Peroxidase/AP Block	5 minutes	5 minutes
Primary Antibody	30 minutes	45 minutes
Secondary Biotinylated Link	10 minutes	Not Applicable
AP or HRP Label	10 minutes	45 minutes
Substrate-Chromogen	5-10 minutes	10 minutes
Counterstaining	Time varies with counterstain	Time varies with counterstain

