



CD45RO

IHC of CD45 RO on an FFPE Tonsil Tissue

Description The CD45 family consists of multiple members that are all products of a single complex gene. Three isoforms of CD45 exist: on B-lymphocytes, where the protein is called B220 (its molecular mass is 220 kDA); on naive T-lymphocytes, where it is called CD45 RA, and on activated and memory T-lymphocytes, where it is called CD45 RO. CD45RO is a single-chain, transmembraneous glycoprotein which represents the low molecular weight isoform of the Leukocyte Common Antigen (LCA). It is expressed on most thymocytes, about 45% of peripheral blood T-cells, virtually all T-cells in skin reactive infiltrates, and the majority of T-cell malignancies. It is also found on a subset of B-cells and on exceptional B-cell Lymphomas.

CD45RO (T-Cell, Pan) antibody reacts with thymocytes and activated T-cells, but only on a subpopulation of resting T-cells. This antibody shows no reactivity with B-cells, making it a good marker for T-cell tumors to be phenotyped. In addition, granulocytes and monocytes are also labeled with this antibody. T-Cell, Pan has been designated as CD45RO at The International Leukocyte Typing Workshop.

Antibody Type	Mouse Monoclonal	Clone	UCLH-1
Isotype	IgG2a/K	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 CD45RO is a mouse 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 5260	Prediluted	Ready-To-Use	3.0 ml
	BSB 5261	Prediluted	Ready-To-Use	7.0 ml
	BSB 5262	Prediluted	Ready-To-Use	15.0 ml
	BSB 5263	Concentrated	1:100-1:500	0.1 ml
	BSB 5264	Concentrated	1:100-1:500	0.5 ml
	BSB 5265	Concentrated	1:100-1:500	1.0 ml
	BSB 5266	Control Slides		5

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

- References**
- Hall PA, et al. *J Clin Path.* 1987;40:151-156
 - Smith SH, et al. *Immunology.* 1986;58:63-70
 - Tworek JA, et al. *Am J Clin Pathol.* 1998;Nov;110(5):582-589

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

