



CD25

IHC of CD25 on an FFPE Tonsil Tissue

Description CD25 is the alpha chain of the IL-2 receptor. It is a Type I transmembrane protein present on activated T-cells, activated B-cells, some thymocytes, myeloid precursors, and oligodendrocytes that associates with CD122 to form a heterodimer that can act as a high-affinity receptor for IL-2. It is expressed in most B-cell neoplasms, some Acute Non-lymphocytic Leukemias, and Neuroblastomas.

Expression of CD25 is a reliable diagnostic tool for distinguishing neoplastic mast-cell aggregates from reactive proliferations, and has, therefore, recently become a minor criterion for the diagnosis of Systemic Mastocytosis (SM). Anti-CD25 antibodies have also been useful in identifying mast cells in skin biopsies in the setting of Urticaria Pigmentosa, which is predictive of Systemic Mastocytosis. Quantitation of regulatory T-cells (Treg) in the setting of hepatocellular carcinoma has been used as an independent predictive factor for tumor recurrence after hepatic resection for HCC. Also, the percentage of tumor-infiltrating CD25+FOXP3+ regulatory T-cells among tumor cells, inside tumor parenchyma and at its periphery are significantly higher in recurrent Cutaneous Melanoma than in Non-recurrent Melanoma.

Antibody Type	Mouse Monoclonal	Clone	4C9
Isotype	IgG2b	Reactivity	Paraffin, Frozen
Localization	Cytoplasmic, Membranous	Control	Mastocytosis, Tonsil, Small Bowel
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 CD25 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 6317	Prediluted	Ready-To-Use	3.0 ml
	BSB 6318	Prediluted	Ready-To-Use	7.0 ml
	BSB 6319	Prediluted	Ready-To-Use	15.0 ml
	BSB 6320	Concentrated	1:25-1:100	0.1 ml
	BSB 6321	Concentrated	1:25-1:100	0.5 ml
	BSB 6322	Concentrated	1:25-1:100	1.0 ml
	BSB 6323	Control Slides		5

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

- References**
- Hahn HP, Hornick JL, *Am J Surg Pathol.* 2007;Nov;31(11):1669-76
 - Hollmann TJ, et al. *Am J Surg Pathol.* 2008;Jan;32(1):139-45
 - Miracco C, et al. *Oncol Rep.* 2007;Nov;8(5):1115-22
 - Siddiqui SA, et al. *Clin Cancer Res.* 2007;Apr;13(7):2075-81

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

