

CD25 (4C9)

For In Vitro Diagnostic Use (IVD)

English: Instructions For Use

Presentation

Anti-CD25 is a mouse monoclonal antibody from supernatant diluted in tris buffered saline, pH 7.3-7.7, with protein base, and preserved with sodium azide.

Applications

According to the World Health Organization classification system, the major diagnostic criterion for bone marrow involvement by Systemic Mastocytosis (SM) is the presence of dense aggregates (>15 cells) of mast cells. Expression of CD25, a low-affinity receptor for interleukin-2 (IL-2), 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 SM. Hahn et al. demonstrated that aberrant staining of Mast cell clusters by anti-CD25 antibody in GI biopsies was essentially diagnostic of SM. Anti-CD25 antibody has 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 of 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, is significantly higher in recurrent cutaneous melanoma than in non-recurrent melanoma.

Reactivity	Paraffin, frozen
Control	Lesions of mastocytosis, small bowel
Visualization	Cytoplasmic, membranous
Stability	Up to 36 months; store at 2-8°C
Isotype	IgG _{2b}

Antibody color does not affect performance

Description	Cat. No.	Dilution/Comments
0.1 ml, concentrate	125M-14	1:10 - 1:50*
0.5 ml, concentrate	125M-15	1:10 - 1:50*
1 ml, concentrate	125M-16	1:10 - 1:50*
1 ml, prediluted	125M-17	Ready to use
7 ml, prediluted	125M-18	Ready to use
Positive control slides	125S	5 slides/pack

- prediluted
 concentrate

Preparation and Pretreatment

1. Cut 3-4 µm section of formalin-fixed paraffin-embedded tissue and place on positively charged slides; dry overnight at 58°C.
2. Deparaffinize, rehydrate, and epitope retrieve; the preferred method is the use of Heat Induced Epitope Retrieval (HIER) techniques using Cell Marque's Trilogy™ in conjunction with a pressure cooker. The preferred method allows for simultaneous deparaffinization, rehydration, and epitope retrieval. Upon completion, rinse with 5 changes of distilled or deionized water.
3. If using HRP detection system, place slides in peroxide block for 10 minutes; rinse. If using AP detection system, omit this step.

Recommended Protocol for Staining at Room Temperature Using CytoScan™ BSA Detection System

1. Apply the antibody and incubate for 30 - 60 minutes; rinse.
2. Apply the link and incubate for 10 minutes; rinse.
3. Apply the label and incubate for 10 minutes; rinse.
4. Apply ample amount of chromogen and incubate for 1 - 10 minutes; rinse.
5. Dehydrate and coverslip.

Recommended Protocol for Staining at Room Temperature Using PolyScan™ Polymer Detection System

1. Apply the antibody and incubate for 30 - 60 minutes; rinse.
2. Apply the PolyScan™ Polymer Rabbit/Mouse Detection System for 30 minutes; rinse.
3. Apply ample amount of chromogen and incubate for 1 - 10 minutes; rinse.
4. Dehydrate and coverslip.

References

1. Hahn HP, Hornick JL. Immunoreactivity for CD25 in gastrointestinal mucosal mast cells is specific for systemic mastocytosis. *Am J Surg Pathol.* 2007 Nov;31(11):1669-76.
2. Hollmann TJ, Brenn T, Hornick JL. CD25 expression on cutaneous mast cells from adult patients presenting with urticaria pigmentosa is predictive of systemic mastocytosis. *Am J Surg Pathol.* 2008 Jan;32(1):139-45.
3. Miracco C, Mourmouras V, Biagioli M, Rubegni P, Mannucci S, Monciatti I, Cosci E, Tosi P, Luzi P. Utility of tumour-infiltrating CD25+FOXP3+ regulatory T cell evaluation in predicting local recurrence in vertical growth phase cutaneous melanoma. *Oncol Rep.* 2007 Nov;18(5):1115-22.
4. Siddiqui SA, Frigola X, Bonne-Annee S, Mercader M, Kuntz SM, Krambeck AE, Sengupta S, Dong H, Cheville JC, Lohse CM, Krco CJ, Webster WS, Leibovich BC, Blute ML, Knutson KL, Kwon ED. Tumor-infiltrating Foxp3-CD4+CD25+ T cells predict poor survival in renal cell carcinoma. *Clin Cancer Res.* 2007 Apr 1;13(7):2075-81.

*The dilutions set forth above are estimates; actual results may differ because of variability in methods and protocols. Validation of antibody performance/protocol is the responsibility of the end user.