

Novocastra™ Liquid Mouse Monoclonal Antibody EZH2 (Enhancer of Zeste Homolog 2 (Drosophila))

Product Code: NCL-L-EZH2

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Human EZH2 (Enhancer of Zeste Homolog 2 (Drosophila)), (85 kD). Region common to isoforms A and B of the protein.
Clone	6A10
Ig Class	IgG1
Antigen Used for Immunizations	Prokaryotic recombinant protein corresponding to a portion of the N-terminus domain of the human EZH2 molecule.
Hybridoma Partner	Mouse myeloma (p3-NS1-Ag4-1).
Preparation	Liquid tissue culture supernatant containing 15 mM sodium azide. Volume as indicated on vial label.
Effective on Frozen Tissue	Not evaluated.
Effective on Paraffin Wax Embedded Tissue	Yes (using heat induced epitope retrieval: see overleaf).
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:200. Heat induced epitope retrieval. 30 minutes primary antibody incubation at 25 °C. Polymer detection recommended. Western Blotting: Typical working dilution 1:25–1:250 (ECL™, Amersham Pharmacia Biotech).
Positive Controls	Immunohistochemistry: Tonsil. Western Blotting: MDA-157, PC3 and SUP-T cell lines (85 kD band).
Staining Pattern	Nuclear.
Storage and Stability	Store liquid antibody at 4 °C. Under these conditions, there is no significant loss in product performance up to the expiry date indicated on the vial label. Prepare working dilutions on the day of use.
General Overview	Polycomb-group proteins (PcG) such as EZH2 (enhancer of zeste homolog 2 (Drosophila)) form multimeric gene repressing complexes involved in axial patterning, hematopoiesis and cell cycle regulation. PcG proteins ensure correct embryonic development by expressing homeobox genes as well as contributing to the regulation of lymphopoiesis. EZH2 expression as detected by IHC has been reported in tonsil where EZH2 expression was associated with Ki-67 centroblasts.
General References	Kattan M. J National Cancer Institute, 95(9): 634–635 (2003). Van Kemenade FJ, Raaphorst FM, Blokzijl T et al. Blood, 97(12): 3896–3901 (2001). Raaphorst Fm, Van Kemenade FJ, Blokzijl T et al. American Journal of Pathology, 157(3): 709–715 (2000).



Instructions for Use

Heat Induced Epitope Retrieval Combined With Polymer Detection For Immunohistochemical Demonstration On Paraffin Sections

1. Cut and mount sections on slides coated with a suitable tissue adhesive.
2. Deparaffinize sections and rehydrate to distilled water.
3. Place sections in 0.5% hydrogen peroxide/methanol for 10 minutes (or use other appropriate endogenous peroxidase blocking procedure). Wash sections in tap water.
4. Heat 1500 mL of the recommended epitope retrieval solution (Citrate based pH 6.0 - Epitope Retrieval Solution unless otherwise indicated overleaf) in a stainless steel pressure cooker until boiling. Cover but do not lock lid.
5. Position slides into metal staining racks (do not place slides close together as uneven staining may occur) and lower into pressure cooker ensuring slides are completely immersed in epitope retrieval solution. Lock lid.
6. When the pressure cooker reaches operating temperature and pressure (after about 5 minutes) start a timer for 1 minute (unless otherwise indicated on the data sheet).
7. When the timer rings, remove pressure cooker from heat source and run under cold water with lid on. DO NOT OPEN LID UNTIL THE INDICATORS SHOW THAT PRESSURE HAS BEEN RELEASED. Open lid, remove slides and place immediately into a bath of tap water.
8. Wash sections once using fresh Tris-Buffered Saline (TBS, pH 7.6) buffer for 5 minutes.
9. Place sections in diluted normal serum (eg NCL-G-SERUM) for 10 minutes.
10. Incubate sections with primary antibody.
11. Wash twice, each time using fresh TBS buffer for 5 minutes.
12. For visualization of the bound primary antibody, follow instructions supplied with the Polymer Detection System.
13. Counterstain with hematoxylin (if required), dehydrate and mount.

** (In most applications, Phosphate Buffered Saline, pH 7.6, can be used instead of TBS, pH 7.6).*

Safety Note

To ensure the correct and safe use of your pressure cooker, PLEASE READ THE MANUFACTURER'S INSTRUCTIONS.