

Novocastra™ Liquid Mouse Monoclonal Antibody DOG-1

Product Code: NCL-L-DOG-1

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Human DOG-1
Clone	K9
Ig Class	IgG2b
Antigen Used for Immunizations	Prokaryotic recombinant protein corresponding to part of the C-terminus of the cytoplasmic domain.
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 with Citrate-based buffer, pH 6.0: see overleaf).
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:100. Heat induced epitope retrieval technique using Citrate-based buffer, pH 6.0. 30 minutes primary antibody incubation at 25 °C. Polymer detection recommended. Technical Note: The use of PBS-based diluents may result in increased background staining.
Positive Controls	Immunohistochemistry: Gastrointestinal stromal tumor (GIST)
Staining Pattern	Membrane and cytoplasmic.
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	DOG-1, a 986 amino acid protein of unknown function, is expressed predominantly on the plasma membrane of gastrointestinal stromal tumors (GISTs) and is rarely expressed in other soft tissue tumors, which, due to appearance, can be confused with GISTs. Immunoreactivity for DOG-1 has been reported to be found in 97.8% of scorable GISTs, including all KIT negative GISTs. Reactivity for DOG-1 has been suggested to aid in the identification of GISTs, including Platelet -Derived Growth Factor Receptor Alpha mutants that fail to express KIT antigen.
General References	Miettinen M and Lasota J. Archives of Pathology and Laboratory Medicine. 130:1466–1478 (2006) Rubin B. Histopathology. 48:83–96 (2006).



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.