Novocastra™ Lyophilized Mouse Monoclonal Antibody CD44 Variant 6

Product Code: NCL-CD44v6

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Epitope encoded by exon v6 on the variant portion of the human CD44 molecule.
Clone	VFF-7
Ig Class	IgG1, kappa
Antigen Used for Immunizations	Prokaryotic recombinant protein expressed in pGEX corresponding to exon variant 6 of the CD44 molecule.
Hybridoma Partner	Mouse myeloma (P3X63Ag8.653).
Preparation	Lyophilized and purified tissue culture supernatant diluted in PBS with 1% BSA containing 15 mM sodium azide. Reconstitute with the volume of sterile distilled water indicated on the vial label.
Effective on Frozen Tissue	Yes. Acetone fixation recommended.
Effective on Paraffin Wax Embedded Tissue	Yes (using the high temperature antigen unmasking technique: see overleaf).
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:50–1:100. 60 minutes primary antibody incubation at 25 °C. High temperature antigen unmasking technique. Standard ABC technique. Western Blotting: Not recommended.
Positive Controls	Immunohistochemistry: Tonsil. Western Blotting: Not recommended.
Staining Pattern	Membrane.
Storage and Stability	Store unopened lyophilized 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. The reconstituted antibody is stable for at least two months when stored at 4 °C. For long term storage, it is recommended that aliquots of the antibody are frozen at -20 °C (frost-free freezers are not recommended). Repeated freezing and thawing must be avoided. Prepare working dilutions on the day of use.
General Overview	CD44 is a widely expressed cell surface glycoprotein that serves as an adhesion molecule in cell-substrate and cell-cell interactions, including lymphocyte homing, hemopoiesis and cell migration. CD44 is a single gene with 20 exons, of which 10 are normally expressed to encode basic CD44 (H-CAM) molecule. The additional 10 exons (v1to v10) are only expressed by alternative splicing of the nuclear RNA and these give rise to the CD44 variants. CD44 variants are differentially expressed by various epithelia.
General References	Anwar F and Wood B L. Modern Pathology. 13 (10): 1121–1127 (2000). Gasbarri A, Martegani M P, Prete F D, et al.. Journal of Clinical Oncology. 17 (11): 3494–3502 (1999). Lipponen P, Aaltoma S, Kosma V-M, et al.. Journal of Pathology. 186: 157–164 (1998). Müller W, Schneiders A, Heider K-H, et al.. Journal of Pathology. 183: 222–227 (1997). Yu Q, Toole B P and Stamenkovic I. Journal of Experimental Medicine. 186 (12): 1985–1996 (1997). Ruiz P, Schwärzler C and Günthert U. BioEssays. 17 (1): 17–24 (1995). Fox S B, Fawcett J, Jackson D G, et al.. Cancer Research. 54: 4539–4546 (1994).

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Instructions for Use

High Temperature Antigen Unmasking Technique 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 unmasking solution (0.01 M citrate buffer, pH 6.0 (or Epitope Retrieval Solution, RE7113) unless otherwise indicated overleaf) until boiling in a stainless steel pressure cooker. 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 unmasking 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 in TBS* buffer (pH 7.6) for 1 x 5 minutes.
9. Place sections in diluted normal serum (or RTU Normal Horse Serum) for 10 minutes.
10. Incubate sections with primary antibody. Use Antibody Diluent RE7133 (where available).
11. Wash in TBS buffer for 2 x 5 minutes.
12. Incubate sections in an appropriate biotinylated secondary antibody.
13. Wash in TBS buffer for 2 x 5 minutes.
14. Incubate slides in ABC reagent (or RTU streptavidin/peroxidase complex).
15. Wash in TBS buffer for 2 x 5 minutes.
16. Incubate slides in DAB or other suitable peroxidase substrate.
17. Wash thoroughly in running tap water.
18. Counterstain with hematoxylin (if required), dehydrate and mount.

Solutions

0.01 M CITRATE BUFFER (pH 6.0) or RE7113 (where available).
Add 3.84 g of citric acid (anhydrous) to 1.8 L of distilled water. Adjust to pH 6.0 using concentrated NaOH. Make up to 2 L with distilled water.

1 mM EDTA (pH 8.0) or RE7116 (where available).
Add 0.37 g of EDTA (SIGMA product code E-5134) to 1 litre of distilled water. Adjust pH to 8.0 using 1.0 M NaOH.

20 mM TRIS/ 0.65 mM EDTA/ 0.005% TWEEN (pH 9.0) or RE7119 (where available).
Dissolve 14.4 g Tris (BDH product code 271197K) and 1.44 g EDTA (SIGMA product code E-5134) to 0.55 L of distilled water. Adjust pH to 9.0 with 1 M HCl and add 0.3 mL Tween 20 (SIGMA product code P-1379). Make up to 0.6 L with distilled water. This is a 10x concentrate which should be diluted with distilled water as required (eg 150 mL diluted with 1350 mL of distilled water).

** In most applications, 10 mM phosphate, 0.15 M NaCl, pH 7.6 (PBS) can be used instead of 50 mM Tris, 0.15 M NaCl, pH 7.6 (TBS).*

Safety Note

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