

Novocastra™ Ready-to-Use Mouse Monoclonal Antibody CD15

Product Code: RTU-CD15

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Human CD15 antigen (3-Fucosyl-N-acetyl-lactosamine).
Clone	BY87
Ig Class	IgM
Antigen Used for Immunizations	Peripheral blood cells from a patient with B cell lymphoma.
Hybridoma Partner	Mouse myeloma (p3-NS1-Ag4-1).
Preparation	Tissue culture supernatant diluted in 5% horse serum in PBS containing 12 mM sodium azide. Volume as indicated on vial label.
Effective on Frozen Tissue	Yes
Effective on Paraffin Wax Embedded Tissue	Yes. The high temperature antigen unmasking technique using 1 mM EDTA (pH 8.0), or trypsin digestion of sections is required. The choice of unmasking technique should be determined by the individual laboratory and is dependent upon fixation methods used.
Recommendations on Use	Immunohistochemistry: Typical working dilution: NEAT. 15 minutes primary antibody incubation at 25 °C when used in conjunction with the Novostain Universal Detection Kit (Ready to Use), code NCL-RTU-D. Recommendations on use will differ if other detection systems are used eg Standard ABC technique. Western Blotting: Not recommended. Not recommended for use on Ventana automated staining systems (Ventana Medical Systems Inc., USA).
Positive Controls	Immunohistochemistry: Small bowel.
Staining Pattern	Membrane and a granular paranuclear zone of labelling in some cell types.
Storage and Stability	Store ready-to-use prediluted liquid antibody at 4 °C. Return to 4 °C immediately after use. Under these conditions, there is no significant loss in product performance up to the expiry date indicated on the vial label.
General Overview	CD15 antigen, also termed X-hapten, is expressed on 90 per cent of circulating human granulocytes, 30 to 60 per cent of circulating monocytes and is absent from normal lymphocytes. CD15 antibodies are reported to recognize the terminal trisaccharide structure GlcNAc which is also referred to as the Lewis x antigen. This structure is found on a variety of glycoproteins and glycolipids at the cell surface.
General References	Hall P A and D'Ardenne A J. Journal of Clinical Pathology. 40: 1298–1304 (1987). Lee F D. Histopathology. 11: 1211–1217 (1987). Dorfman R F, Gatter K C, Pulford K A, et al.. American Journal of Pathology. 123: 508–519 (1986).



Instructions for Use

High Temperature Antigen Unmasking Technique Followed by Trypsin Digestion 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 - see other Epitope Retrieval Solutions in the range) 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. Place slides in pre-heated distilled water to bring the sections to 37 °C for a minimum of 5 minutes.
9. Incubate sections in pre-heated Trypsin solution at 37 °C for 30 seconds.
10. Rinse sections in running tap water.
11. Proceed with immunohistochemistry protocol.
12. Wash sections in TBS* buffer (pH 7.6) for 1 x 5 minutes.
13. Place sections in diluted normal serum (or RTU Normal Horse Serum) for 10 minutes.
14. Incubate sections with primary antibody. Use Antibody Diluent RE7133 (where available).
15. Wash in TBS buffer for 2 x 5 minutes.
16. Incubate sections in an appropriate biotinylated secondary antibody.
17. Wash in TBS buffer for 2 x 5 minutes.
18. Incubate slides in ABC reagent (or RTU streptavidin/peroxidase complex).
19. Wash in TBS buffer for 2 x 5 minutes.
20. Incubate slides in DAB or other suitable peroxidase substrate.
21. Wash thoroughly in running tap water.
22. Counterstain with hematoxylin (if required), dehydrate and mount.

Solutions

Trypsin Solution

**Trypsin containing chymotrypsin should always be used. The enzyme activities can vary from a supplier and between batches. Such variations may affect the incubation time required.*

Preheat the following to 37 °C using a water bath:

- (i) 200 mL of TBS
- (ii) 200 mL of distilled water.

Dissolve 0.2 g Trypsin 250 and 0.2 g Calcium Chloride in the 200 mL of TBS.

Once the Trypsin solution is at 37 °C, pH to 7.8 with 1 M sodium hydroxide.

0.01 M Citrate Buffer (pH 6.0) or RE7113 (where available).

Add 3.84 grams 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 L of distilled water. Adjust pH to 8.0 using 1.0 M NaOH.

20 mM TRIS/0.65 mM EDTA/0.0005% 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, 10mM 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 THE MANUFACTURER'S INSTRUCTIONS.