Novocastra™ Lyophilized Mouse Monoclonal Antibody CD54 (ICAM-1)

Product Code: NCL-CD54-307

Intended Use
FOR RESEARCH USE ONLY.

Specificity
Human CD54 antigen (ICAM-1).

Clone
23G12

Ig Class
IgG1

Antigen Used for Immunizations
Prokaryotic recombinant protein corresponding to a region of the external domain of the human CD54 (ICAM-1) molecule.

Hybridoma Partner
Mouse myeloma (p3-NS1-Ag4-1).

Preparation
Lyophilized tissue culture supernatant containing 15 mM sodium azide. Reconstitute with 1 mL or 0.1 mL of sterile distilled water as indicated on vial label.

Effective on Frozen Tissue
No

Effective on Paraffin Wax Embedded Tissue
Yes (using the high temperature antigen unmasking technique: see overleaf).

Recommendations on Use
Immunohistochemistry: Typical working dilution 1:25–1:50. High temperature antigen unmasking technique. 60 minutes primary antibody incubation at 25 °C. 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
The CD54 (ICAM-1) is an integral membrane glycoprotein of 85 to 110 kD with seven potential N-linked glycosylation sites. The antigen is expressed on monocytes and endothelial cells. Expression of the CD54 glycoprotein can be induced or upregulated on many cell types including B and T lymphocytes, thymocytes, fibroblasts, keratinocytes and epithelial cells. The CD54 antigen is important in mediating immune and inflammatory responses. It mediates the adhesion of T cells with antigen-presenting cells and is involved in T cell to T cell and T cell to B cell interactions.

General References
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 27119K) 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.