

Novocastra™ Ready-to-Use Mouse Monoclonal Antibody CD4

Product Code: RTU-CD4-1F6

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| Intended Use | FOR RESEARCH USE ONLY. |
| Specificity | Human CD4 antigen. |
| Clone | 1F6 |
| Ig Class | IgG1 |
| Antigen Used for Immunizations | Prokaryotic recombinant protein corresponding to the external domain of the CD4 molecule. |
| 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 | No |
| Effective on Paraffin Wax Embedded Tissue | Yes (using 1 mM EDTA (pH 8.0) unmasking solution combined with the high temperature antigen unmasking technique: see overleaf). |
| Recommendations on Use | <p>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. Technical note: The use of H₂O₂ to block endogenous peroxidase has a detrimental effect on the epitope recognised by RTU-CD4-1F6. Solutions containing 1% or greater H₂O₂ can dramatically REDUCE the level of staining achievable. Blocking after unmasking can also lead to negative results. Therefore, it is recommended that endogenous peroxidase should be blocked BEFORE unmasking with 0.5% H₂O₂/methanol for 10 minutes. If this recommendation is not followed, staining intensity may be reduced.</p> <p>Western Blotting: Not recommended.</p> <p>Not recommended for use on Ventana automated staining systems (Ventana Medical Systems Inc., USA).</p> |
| Positive Controls | Immunohistochemistry: Tonsil |
| Staining Pattern | Membrane |
| 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 | The CD4 molecule (T4) is a single chain transmembrane glycoprotein with a molecular weight of 59kD. The CD4 antigen is found on a T cell subset (helper/inducer) representing 45 per cent of peripheral blood lymphocytes. It is also present on 80 per cent of thymocytes and at a lower level on monocytes. |
| General References | <p>Izban K F, Hsi E D and Alkan S. Modern Pathology. 11 (10): 978–982 (1998).</p> <p>Williamson S L, Steward M, Milton I, et al.. American Journal of Pathology. 152 (6): 1421–1426 (1998).</p> <p>Arthos J, Deen K C, Chaikin M A, et al.. Cell. 57: 469–481 (1989).</p> <p>Petersen A and Seed B. Cell. 54: 65–72 (1988).</p> <p>Gay D, Maddon P, Sekaly R, et al.. Nature. 328: 626–629 (1987).</p> <p>Dalgleish A G, Beverly P C L, Clapham P R, et al.. Nature. 312: 763–766 (1984).</p> <p>Stein H, Lennert K, Feller A C, et al.. Advances in Cancer Research. 42: 67–147 (1984).</p> |



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.