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TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Affinity Purified Anti-Mouse CD13 (Aminopeptidase N)

CL89141
Lot:

DESCRIPTION: Cedarlane's CL89141 is a useful marker for the identification of aminopeptidase N positive macrophages, interdigitating cells and dendritic cells. It is also useful for *in vitro* monitoring of M-CSF stimulated bone marrow cell cultures, as the antigen is gradually expressed with macrophage development. Expression of the ER-BMDM1 antigen rises after the monocytic stage of differentiation: bone marrow cells and peripheral blood monocytes are ER-BMDM1 negative, whereas virtually all thioglycollate elicited peritoneal exudate macrophages bind the antibody. The CD designation is based on similarity in molecular and functional characteristics.

Antigen Distribution:

Isolated Cells: The antigen is present on the majority of isolated dendritic cells of the spleen and lymph node. Over 80% of thioglycollate elicited peritoneal exudate macrophages also express the ER-BMDM1 related antigen. It is absent from freshly isolated bone marrow and blood cells.

Lymphoid organs: macrophages surrounding small blood vessels, interdigitating cells, subpopulation of macrophages in the T cells areas, capsular and medullary cord macrophages in lymph nodes.

Non-lymphoid organs: subpopulation of macrophages (mainly in connective tissues) and dendrocytes, structures positive for aminopeptidase such as the brush border of the small intestine, bile canaliculi in the liver and tubuli and glomeruli in the kidney or type II pneumocytes in the lung. Kupffer cells are negative.

SPECIFICITY: Reacts with mature mouse macrophages. Not tested with other species. The antigen is a 160 kDa membrane associated protein which shows aminopeptidase N activity. It is homologous to the human CD13 marker.

CLONE: ER-BMDM1

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For more information or to place an order please contact...

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IMMUNOGEN: Cultured mouse monocytes, day 7.

ISOTYPE: Rat $IgG2_a$

PRESENTATION: 100 µg lyophilized affinity purified product. Reconstitute by adding 0.5 ml distilled water. This stock solution contains 0.2 mg/ml IgG, PBS pH 7.2, 10 mg/ml BSA and 0.01% thimerosal.

STORAGE/STABILITY: Lyophilized product stable for 1 year at 4°C. Stock solution and aliquots are stable for 1 year at -20C. Avoid multiple freeze/thaw cycles.

APPLICATIONS: Suitable for use in FACS and immunohistochemistry (IHC).

Approximate working dilutions for IHC:
Frozen sections: 1 ug/ml (1:200)*
Paraffin sections: 40 ug/ml (1:5)*; pretreatment not necessary

*Optimal dilutions should be determined by the end user

Suggested positive control: Mouse spleen

STANDARD PROCEDURE FOR TISSUE SECTIONS AND SMEARS USING A PEROXIDASE CONJUGATED SECONDARY ANTIBODY

1. Reconstitution: Add the amount of sterile distilled water to the vial of antibody, close the vial and agitate gently from time to time, keep at room temperature (20°C) for 15 minutes before use.
2. Storage: Make aliquots for deep freezing (shock freezing with dry ice / acetone or with liquid nitrogen) at –70°C or store the stock solution in the original vial at 4°C. Pipette under sterile conditions and never let the stock solution stand for long at room temperature. Do not keep working dilutions.
3. Fixation: Fix your cryostat sections or biopsied tissues on gelatine-chrom alum coated slides with acetone for 10 minutes and dry quickly in the air stream (ventilator, do not heat).
4. Wash: Gently rinse your slides with physiological PBS buffer solution (0.15M, pH 7.2) 3 x 1 sec.
5. Incubate: 20 minutes in PBS (same buffer solution as in #4) containing 0.15M NaN_3 and 500ul H_2O_2 at room temperature (20°C) (=blocking of endogenous peroxidase activity).
6. Wash: 3 x 3 minutes with PBS (same solution as in #4) containing 0.05 Tween 20.
7. Incubate: 15 minutes with PBS (same buffer solution as in #4) containing 50% normal serum of the same species as your second antibody conjugate (= avoids non-specific conjugate binding).
8. Wash: 3 x 3 minutes with PBS (same buffer solution as in #4).
9. Incubate: 1 hour at 37°C with appropriate dilution of #CL89141 in a humid chamber.
10. Wash: 2 x 3 minutes with physiological PBS (same buffer solution as in #4).
11. Incubate: 1 hour at 37°C with your conjugate (= second antibody = peroxidase conjugated anti-mouse or rat IgG) in a humid chamber.
12. Wash: 2 x 3 minutes with physiological PBS (same buffer solution as in #4).
13. Incubate: 12 minutes at room temperature (20°C) in a humid chamber in the dark with substrate. Substrate: 5 ml stock solution of AEC (= 0.4g AEC/100 ml DMF) in 100ml sodium acetate buffer solution (0.1M, pH 4.9) + 40ul H_2O_2; AEC = 3 amino-9-ethyl-carbazole, DMF = Dimethylformamide
14. Wash: Gently 2 x 1 seconds with distilled water.
15. Wash; Gently rinse for 10 seconds in sodium acetate buffer solution (same buffer solution as in #13).

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COUNTERSATIN: with Mayers-hemalum or Methyl green

RESULTS; specific staining = red to brown coloured cells

REFERENCES:

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2. Allaerts, W. et al. J. Neuroimmunology. **78**: 184-197 (1997).
3. Leenen, P.J.M. et al. J. Immunol. **160**:2166-73 (1998).

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JCR 06/07/06