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AM39008PU-N Monoclonal Antibody to CD4 - Aff - Purified

Alternate names: T-cell surface antigen T4/Leu-3, T-cell surface glycoprotein CD4

Quantity: 1 ml

Concentration: 0.1 mg / ml

Background: CD4 plays a role in the recognition of foreign antigens presented to T cells by MHC class II molecules. Furthermore, this antigen acts as a receptor for HIV-1 by binding the viral protein gp120. The CD4 antigen is present on most thymocytes and a subpopulation of peripheral blood T cells, called T helper cells (Th). In addition, CD4 is expressed on monocytes and weak on macrophages.

Uniprot ID: P01730

NCBI: NP_000607

GeneID: 920

Host / Isotype: Mouse / IgG2a

Clone: Edu-2

Format:
State: Liquid purified Ig fraction
Purification: Affinity chromatography
Buffer System: 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.3, 0.2% BSA, 0.09% sodium azide

Applications:
- Flow cytometry: The CD4 antibody (clone Edu-2) is used in routine blood testing for CD4+ cells and CD4/CD8 ratios (e.g. HIV/AIDS patients) or as part of panels for the detection and differentiation of certain T cell leukemias.
CD4 is also used in studies of functional activity of Th-cells in bacterial and viral infections, development of auto-immune diseases, transplant rejection, immune protection in response to allergens or allergenic reactivity (see "Protocols" below).
- Immunofluorescence / Immunohistochemistry using frozen and paraffin embedded tissue sections.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: Anti CD4 (Edu-2) recognizes the CD4 antigen (a 55 kD glycoprotein).
Species: Human.
Other species not tested.

Add. Information:
1. Conjugates with brighter fluorochromes, like PE and APC, will have a greater separation than those with dyes like FITC. When populations overlap, the percentage of positive cells using a selected marker can be affected by the choice of fluorescent label.
2. Use of monoclonal antibodies in patient treatment can interfere with antigen target recognition by this reagent. This should be taken into account when samples are analyzed from patients treated in this fashion.
3. Reagent data performance is based on EDTA-treated blood. Reagent performance can be affected by the use of other anticoagulants.

For research and in vitro use only. Not for diagnostic or therapeutic work.
Material Safety Datasheets are available at www.acris-antibodies.com or on request.

Storage:

Store the antibody undiluted at 2-8°C.
Shelf life: one year from despatch.

General Readings:

1. Carrière, D., et al., 1995, In: Leukocyte Typing V: 475-476. S.F. Schlossman, L. Boumsell, W., et al., eds. Oxford University Press, New York
2. Piatier-Tonneau, D., et al.,1995, In: Leukocyte Typing V: 476-478. S.F. Schlossman, L. Boumsell, W. Gilks, et al., eds. Oxford University Press, New York
3. Kaneoka H, Perez-Rojas G, Sasasuki T, Benike CJ, Engleman EG. Human T lymphocyte proliferation induced by a pan-T monoclonal antibody (anti-Leu 4): heterogeneity of response is a function of monocytes. J Immunol. 1983 Jul;131(1):158-64. PubMed PMID: 6408167.
4. Lanier LL, Allison JP, Phillips JH. Correlation of cell surface antigen expression on human thymocytes by multi-color flow cytometric analysis: implications for differentiation. J Immunol. 1986 Oct 15;137(8):2501-7. PubMed PMID: 3531334.
5. Knowles, R.W., 1986. In: Leukocyte Typing II : 259-288; E.L. Reinhert, B.F. Haynes, L.M. Nadler/ and I.D. Bernstein, eds. Springer-Verlag, New York
6. Friedrich W, O'Reilly RJ, Koziner B, Gebhard DF, Good RA, Evans RL. T-lymphocyte reconstitution in recipients of bone marrow transplants with and without GVHD: imbalances of T-cell subpopulations having unique regulatory and cognitive functions. Blood. 1982 Apr;59(4):696-701. PubMed PMID: 6800422.
7. Allison JP, Lanier LL. Structure, function, and serology of the T-cell antigen receptor complex. Annu Rev Immunol. 1987;5:503-40. PubMed PMID: 2439105.

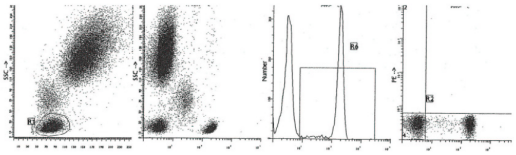
Protocols:

Flow cytometry method for use with purified monoclonal antibodies

1. Add 100 µl of EDTA-treated blood (i.e. approx. 10e6 leukocytes) to a 5 ml reagent tube. The content of one tube is sufficient to perform one test.
2. Add to each tube 10 µl of purified monoclonal antibody. (Appropriate mouse Ig isotype control samples should always be included in any labeling study).
Vortex the tube to ensure thorough mixing of antibody and cells.
3. Incubate the tube for 15 minutes at room temperature in the dark.
4. Wash the labeled cells by adding 2 ml of PBS containing 0.001% (v/v) Heparin, vortexing and centrifuging (2 min 1000 x g) and discard the supernatant.
5. Add 50 µl of appropriate dilution of F(ab)2 Rabbit Anti Mouse IgG fluorescent conjugate (e.g.FITC or R-PE) in PBS containing 0.001% (v/v) Heparin to the tube. It is recommended that the tube is protected from light.
6. Mix by vortexing and incubate for 15 minutes at room temperature in the dark.
7. Add 100 µl of a lyse reagent and mix immediately.
8. Incubate for 10 minutes at room temperature in the dark.
9. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.
10. Centrifuge the labeled cell suspension for 2 minutes at 1000 x g.
11. Remove the supernatant and resuspend the cells in 200 µl of PBS.
12. Analyze by flow cytometry within four hours (alternatively, the cells may be fixed by 0.05% of formaline in buffered saline for analysis the next day. Some antigens are readily destroyed upon fixation and this should be taken into account when using this alternative).

Pictures:

Staining with clone Edu-2 (CD4) monoclonal antibodies is illustrated by flow cytometry analysis of normal blood cells. Indirect staining was performed using 10 µl of the purified monoclonal antibody with RaM FITC conjugate and 100 µl blood sample. Testing by flow cytometry using a 'lyse-wash' method on whole blood from healthy donors showed the following values expressed in terms of % of the total lymphocyte count: Product code: AM39008PU-N (anti CD4 purified) Mean % positive: 45,36 S.D.: 5,20 % CV: 11,46



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