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Product datasheet for AM39008RP-N

CD4 Mouse Monoclonal Antibody [Clone ID: EDU-2]

Product data:

Product Type:	Primary Antibodies
Clone Name:	EDU-2
Applications:	FC
Recommended Dilution:	- 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. The reagent is effectively formulated for direct immunofluorescent staining (see "Protocols" below).
Reactivity:	Human
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Specificity:	Anti CD4 (Edu-2) recognizes the CD4 antigen (a 55 kD glycoprotein). 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: AM39008RP-N (anti-CD4 PE) Mean % positive: 47,18 S.D.: 5,20 % CV: 11,02
Formulation:	0.01 M sodium phosphate, 0.15 M NaCl, pH 7.3, 0.2% BSA, 0.09% sodium azide Label: PE State: Liquid purified Ig fraction Label: (R-Phycoerythrin)
Purification:	Affinity chromatography
Conjugation:	PE
Storage:	Store the antibody undiluted at 2-8°C. This product is photosensitive and should be protected from light.

Stability: Shelf life: one year from despatch.

Gene Name: CD4 molecule

Database Link: Entrez Gene 920 Human
P01730

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.

Synonyms: T-cell surface antigen T4/Leu-3

Note: 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.

Protocol: Flow cytometry method for use with labeled (FITC, R-PE, APC, PerCP or PerCP-Cy5.5) 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 labeled 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. Add 100 µl of a lyse reagent.
5. Incubate for 10 minutes at room temperature in the dark.
6. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.
7. Centrifuge the labeled cell suspension for 2 minutes at 1000 x g.
8. Remove the supernatant and resuspend the cells in 200 µl of PBS.
9. 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).

Flow cytometry method for use with dual and triple combinations
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.
For combinations with anti-kappa and/or anti-lambda Ig see**Application note** below.
2. Add to each tube 20 µl of labeled monoclonal antibody combination.
(Appropriate mouse Ig isotype control samples should always be included in any labeling

study).
3. Vortex the tube to ensure thorough mixing of antibody and cells.
4. Incubate the tube for 15 minutes at room temperature in the dark.
5. Add 100 µl of a lyse reagent and mix immediately.
6. Incubate for 10 minutes at room temperature in the dark.
7. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.
8. Centrifuge the labeled cell suspension for 2 minutes at 1000 x g.
9. Remove the supernatant and resuspend the cells in 200 µl of PBS.
10. 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).

Application note for anti-kappa and/or anti-lambda Ig combinations
Add 2 ml of PBS containing 0.001% (v/v) Heparin (prewarmed to 37°C) to the cell suspension
Vortex, centrifuge (2 min at 300x g) and discard the supernatant.
Repeat this step twice.
Resuspend the pelleted blood cells in 100 µl PBS, pH 7.2, containing 0.001% (v/v) Heparin.

Protein Families: Adult stem cells, Druggable Genome, ES Cell Differentiation/IPS, Induced pluripotent stem cells, Transmembrane

Protein Pathways: Antigen processing and presentation, Cell adhesion molecules (CAMs), Hematopoietic cell lineage, Primary immunodeficiency, T cell receptor signaling pathway