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Product datasheet for AM39011RP-N

CD8A Mouse Monoclonal Antibody [Clone ID: MCD8]

Product data:

Product Type:	Primary Antibodies
Clone Name:	MCD8
Applications:	FC
Recommended Dilution:	Flow cytometry: The reagent is effectively formulated for direct immunofluorescent staining (see "Protocols" below).
Reactivity:	Human
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Specificity:	The antibody detects the CD8 molecule, expressed as a heterodimer of CD8a (32-34 kD)and CD8b (32-34 kD) glycoproteins. Clone MCD8 is commonly used in routine immunophenotyping, the determination of CD4/CD8 ratios in HIV/AIDS patients and aids in the identification of T cell leukemias (common T-ALL or mature T-ALL)s. MCD8 also distinguishes between chronic B and T cell lymphoid leukemias. 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: AM39011RP-N (anti-CD8 PE) n: 10 Mean % positive: 31,53 S.D.: 4,58 % CV: 14,52
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.

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Stability: Shelf life: one year from despatch.

Gene Name: CD8a molecule

Database Link: Entrez Gene 925 Human
P01732

Background: CD8 acts as a co-receptor with the TcR in recognizing antigens presented by MHC Class I and plays a role in the T cell-mediated immune response. The CD8 antigen is present on most tymocytes, T cytotoxic/suppressor cells and a subpopulation of NK cells.

Synonyms: CD8 alpha chain, CD8A, MAL

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.

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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, Secreted Protein, Transmembrane

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

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