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Product datasheet for AM39007FC-N

CD2 Mouse Monoclonal Antibody [Clone ID: B-E2]

Product data:

Product Type:	Primary Antibodies
Clone Name:	B-E2
Applications:	FC, IF
Recommended Dilution:	- Flow cytometry: for analysis of blood and bone marrow samples. CD2 antibody is applied in flow cytometry for the quantification of the total T-cell population in blood and for the identification of CD2 positive cells in tissue sections. It has also been applied in the analysis of NK cell populations. CD2 antibodies may also be used for the elimination or quantitative isolation of T cells by flow cytometry or magnetic particles. The reagent is effectively formulated for direct immunofluorescent staining (see also "Protocols" below). - Immunofluorescence: using cytospots or frozen tissue sections.
Reactivity:	Human
Host:	Mouse
Isotype:	IgG2b
Clonality:	Monoclonal
Specificity:	Clone B-E2 reacts specifically with a 45-50 kD single chain transmembrane glycoprotein, also known as the LFA-2, the sheep erythrocyte receptor or CD2 antigen.
Formulation:	0.01 M sodium phosphate, 0.15 M NaCl, pH 7.3, 0.2% BSA, 0.09% sodium azide Label: FITC State: Liquid purified Ig fraction
Conjugation:	FITC
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:	CD2 molecule
Database Link:	Entrez Gene 914 Human P06729

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Background: The CD2 antigen plays a role in T cell signaling and in lymphocyte adhesion. The major ligand for the extracellular portion of human CD2 is CD58 (LFA3). CD2 is present on all human peripheral T-lymphocytes and a fraction of the NK cell (large granular lymphocyte) population.

Synonyms: SRBC, Erythrocyte receptor, LFA-2, LFA-3 receptor, Rosette receptor

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.

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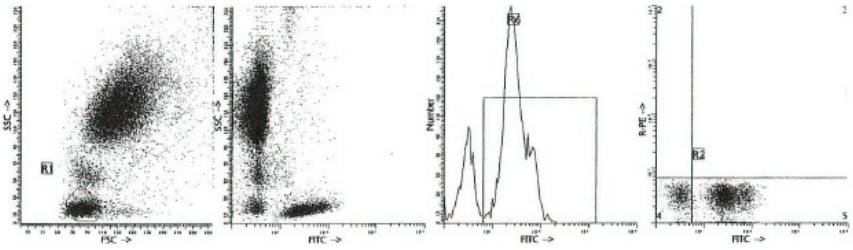
7. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.
8. Centrifuge the labeled cell suspension for 2 minutes at 1000 x g.
9. Remove the supernatant and resuspend the cells in 200 µl of PBS.
10. Analyze by flow cytometry within four hours (alternatively, the cells may be fixed by 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: Druggable Genome, Transmembrane

Protein Pathways: Cell adhesion molecules (CAMs), Hematopoietic cell lineage

Product images:



Staining with clone B-E2 is illustrated by flow cytometry analysis of normal blood cells. Direct staining was performed using 10 µl of the FITC-conjugated antibody with 100 µl blood sample. The antibody was tested by flow cytometry using a 'lyse-wash' method on whole blood from healthy donors. Obtained data support the premise that these reagents are equivalent in their reactivity with peripheral blood lymphocytes. Values are expressed in terms of % of the total lymphocyte count: Product code: AM39007FC-N Mean % positive: 78, 61 S.D.: 5, 68 % CV: 7, 22

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