

Product datasheet for **AM39009FC-N**

CD5 Mouse Monoclonal Antibody [Clone ID: MCD5]

Product data:

Product Type:	Primary Antibodies
Clone Name:	MCD5
Applications:	FC, IF
Recommended Dilution:	MCD5 is used in the identification and localization of T cells in tissue and the diagnosis of T cell lymphomas and of B cell lymphocytic lymphomas of CLL types. - Flow cytometry: for analysis of blood and bone marrow samples. Used in flow cytometry for the enumeration of T cells and CD5 positive B cells in peripheral blood. The reagent is effectively formulated for direct immunofluorescent staining (see "Protocols" below). - Immunofluorescence using cytopspots or frozen tissue sections.
Reactivity:	Human
Host:	Mouse
Isotype:	IgG2b
Clonality:	Monoclonal
Specificity:	MCD5 recognizes a 67 kD antigen on human T cells. Other species not tested.
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 protein
Purification:	Affinity chromatography
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:	CD5 molecule
Database Link:	Entrez Gene 921 Human P06127



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Background:

CD5 appears to be a relatively late marker during B cell differentiation. CD5 expression is thought to be absent on surface Ig negative B-lineage cells but appears on IgM+ cells in both fetal liver and bone marrow. It co-precipitates with the T cell receptor and, in particular with Lck.

A role for CD5 in signal transduction is postulated based on stimulatory effects of CD5 monoclonal antibodies. CD5 antigen is phosphorylated on tyrosine residues on T cell activation. There is evidence that CD5 plays a role in thymocyte selection, as well as a role in cell-cell recognition. Recently CD5 on B cells has been shown to be an endogenous ligand selective for B-cell surface IgFR (framework region) sequences. Interaction of surface Ig with CD5, other endogenous antigens or (in mucosal sites) exogenous superantigens can provide B cells with continual stimulation and might prevent their elimination from the immune system. In addition, B cell superantigens, e.g. Staphylococcus aureus Cowan strain 1, may contribute to the pathogenesis of autoimmune diseases and malignancies.

Synonyms:

CD5, LEU1

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 μ l 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 μ l 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 μ l 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.

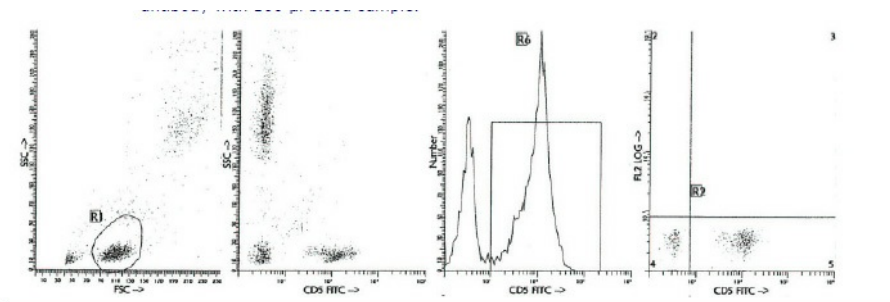
Repeat this step twice.

Resuspend the pelleted blood cells in 100 μ l PBS, pH 7.2, containing 0.001% (v/v) Heparin.

Protein Families: Druggable Genome, Transmembrane

Protein Pathways: Hematopoietic cell lineage

Product images:



Staining with clone MCD5 (CD5) monoclonal antibody is illustrated by flow cytometry analysis of normal blood cells. Direct staining was performed using 10 μ l of the FITC-conjugated antibody with 100 μ l 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: AM39009FC-N (anti-CD5 FITC) Mean % positive: 72, 61 S.D.: 5, 10 % CV: 7,03