

AM39014AC-N**Monoclonal Antibody to CD19 - APC**

Alternate names:	B-cell marker, B-lymphocyte surface antigen B4, Differentiation antigen CD19, Leu-12
Quantity:	100 Tests
Concentration:	Labelled antibody: 100 tests/ml Pure antibody: 0.1 mg/ml (100 tests/ml)
Background:	Progenitor B cells mature in bone marrow and subsequently appear as mature CD19+ B cells in blood. After contact with foreign antigens and the appropriate T cell help, they may further differentiate to specific antibody-producing plasma cells. The function of the CD19 molecule is related to signal transfer and is involved in regulation of B cell proliferation. CD19 is considered to be a characteristic B cell marker and therefore commonly used in routine immunophenotyping. Detection of CD19 expressing cells is important in the diagnosis of leukemic precursor B cells (pre-B ALL), mature B cells (B ALL), and plasma cells. Distinction between subtypes of these (acute) leukemias can be performed using CD19 antibodies together with monoclonal antibodies to cytoplasmic or membrane Ig. A large number of B cell disorders can be effectively characterized by expression of CD19 and one or more additional antigens. One example is hairy cell leukemia (HCL), which shows specific expression of CD11c, CD19, CD20 and CD103. The combination CD103/CD19 is an important tool for diagnosis of HCL. Other valuable antibody combinations for distinction between different leukemias using CD19 antibodies are CD5/CD19 (e.g. Chronic Lymphatic Leukemia); CD10/CD19 (common ALL and pre-B ALL) and CD19/cyCD79a (Acute B cell leukemia). CD19 may also be expressed on follicular dendritic cells.
Uniprot ID:	P15391
NCBI:	NP_001171569
GeneID:	930
Host / Isotype:	Mouse / IgG1
Recommended Isotype Controls:	SM10APC (for use in human samples)
Clone:	HD37

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 Label: APC – <u>Cat. No. Label EX-max (nm) / EM-max (nm):</u> AM39014AC-N APC 595, 633, 635, 647 / 660 AM39014FC-N FITC 488 / 519 AM39014RP-N PE 488, 532 / 578 AM39014PP-N PerCP 488, 532 / 678 AM39014PC5-N 488, 532 / 695 AM39014PU-N Pure . /
Applications:	Anti-CD19 (clone HD37) can be applied in Flow Cytometry and in Immunohistochemistry using Frozen tissue sections. Flow Cytometry: please see "Protocols" below. Labelled reagent is effectively formulated for direct immunofluorescent staining. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Specificity:	Clone HD37 is specific for CD19, it recognizes a 95 kD transmembrane 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: AM39014AC-N (anti-CD19 APC) n: 9 Mean % positive: 9,16 S.D.: 1,95 % CV: 21,31 ----- Product code: AM39014FC-N (anti-CD19 FITC) n: 9 Mean % positive: 10,80 S.D.: 2,14 % CV: 19,79 ----- Product code: AM39014RP-N (anti-CD19 PE) n: 9 Mean % positive: 10,57 S.D.: 2,06 % CV: 19,49 ----- 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.

Storage:

Store the antibody undiluted at 2-8°C.
Fluorochrome labelled product is photosensitive and should be protected from light.
Shelf life: one year from despatch.

General Readings:

1. Poppema, S. and Visser, L., In: Monoclonal antibodies in the Characterization of Lymphomas and the Diagnosis of Disease; Proc. of the 9th Biotest Symposium, Institute of Education, London, 1987. Sonneborn H.H. and Tills D. Eds
2. Moldenhauer, G., et al., In Leucocyte Typing II Human B Lymphocytes, 1986, 61-67, E.L. Reinherz, B.F. Haynes, L.M. Nadler, and I.D. Bernstein eds. (Springer-Verlag, New York)
3. Meeker TC, Miller RA, Link MP, Bindl J, Warnke R, Levy R. A unique human B lymphocyte antigen defined by a monoclonal antibody. Hybridoma. 1984 Winter;3(4):305-20. PubMed PMID: 6441771.
4. Loken MR, Shah VO, Dattilio KL, Civin CI. Flow cytometric analysis of human bone marrow. II. Normal B lymphocyte development. Blood. 1987 Nov;70(5):1316-24. PubMed PMID: 3117132.
5. Rothe G, Schmitz G. Consensus protocol for the flow cytometric immunophenotyping of hematopoietic malignancies. Working Group on Flow Cytometry and Image Analysis. Leukemia. 1996 May;10(5):877-95. PubMed PMID: 8656686.
6. Leucocyte Typing IV 1990, Knapp, W. et al, eds. Oxford University Press.

Protocols:

Flow cytometry method for use with labelled (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 labelled monoclonal antibody. (Appropriate mouse Ig isotype control samples should always be included in any labelling 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 labelled 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 labelled 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 labelled 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.

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 labelling 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 labelled 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 labelled 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 HD37 (CD19) monoclonal antibodies is illustrated by flow cytometry analysis of normal blood cells. Direct staining was performed using 10 µl of the PE - conjugated antibody with 100 µl blood sample.

