Dako

Monoclonal Mouse Anti-Human CD19/RPE, Clone HD37

Code R0808

INTENDED USE

For *In Vitro* Diagnostic use.

Monoclonal Mouse Anti-Human CD19 RPE-conjuagted, Clone HD37 has been developed for use in flow cytometry for the analysis of B cells. This reagent allows simultaneous detection and quantification of CD19-positive cells (B cells) in normal and pathological conditions such as immunodeficiency disorders. It is one component of the suggested monoclonal antibody (mAb) combination for routine immunophenotyping of lymphocytes in peripheral blood (1-3).

SUMMARY AND EXPLANATION

The lymphocyte population of white blood cells (WBCs) consists essentially of three subpopulations, T cells (thymus derived), B cells (bone marrow derived) and natural killer cells. These subpopulations are identified by cell surface antigens and biological function.

Anti-CD19, HD37 (4), reacts with a 90kDa polypeptide which was designated CD19 at the Second International Workshop on Human Leucocyte Differentiation Antigens (Boston, 1984). The CD19 gene has recently been cloned, and the antigen has been shown to be a transmembrane polypeptide with at least two extracellular immunoglobulin-like domains (5). The intracytoplasmic domain bears some resemblance to proteins encoded by Epstein-Barr virus and by the *IMT-1* oncogene (6).

The CD19 antigen appears early during B lymphocyte maturation, probably at the pro B lymphocyte stage (7, 8). It then persists throughout B lymphocyte maturation and is lost shortly before the terminal plasma cell stage (7). The antigen has a possible role in the regulation of B lymphocyte proliferation and differentiation (8, 10). Cross-blocking studies indicate that all antibodies in the CD19 cluster studied at the above workshop define a single epitope (11).

Immunophenotyping of lymphocytes is widely applied for diagnosis of immunodeficiencies (12, 13) Anti-CD19/RPE is one of the reagents utilized when performing immunophenotyping of lymphocytes.

PRINCIPLE OF PROCEDURE

Anti-CD19/RPE, HD37, is a monoclonal antibody to the CD19 epitope, conjugated with R-phycoerythrin (RPE). The conjugate can be detected by light excitation of the fluorochrome. When bound to the specific B cell epitope, that light emission allows measurement of the lymphocyte subset by flow cytometry. Cells that have bound antibody emit fluorescence read at 570 nm, which displays orange-red.

Subpopulations of lymphocytes may be stained with fluorochrome conjugated antibody and evaluated in peripheral blood specimens. Contaminating red blood cells (RBCs) are lysed, or mononuclear cells can be separated from other blood components by a density gradient cell separation method (Ficoll) and stained with the fluorochrome conjugated antibody prior to analysis. A subpopulation of WBCs are selected for assessment based upon cell morphology.

The B cell count is usually expressed as a percent of lymphocytes. When the sample is analyzed on a flow cytometer, percent positive lymphocytes can be determined directly by excluding nonlymphocytic cells from the analysis gate on the basis of size and internal complexity. Because each flow cytometer has different operating characteristics, each laboratory must determine its optimal operating procedure.

REAGENT PROVIDED

PRIMARY ANTIBODY

Purified mouse anti-human CD19, conjugated with R-phycoerythrin is present in 0.05 M Tris/HCl buffer, pH 7.2, 0.1 M NaCl, 0.015 M NaN_3, stabilized with 1% carrier protein.

CD19 Clone	*Immunogen:*	Hairy cell leukemia cells
	Source:	Tissue culture supernatant
	Clone/Ref.:	HD37
	Isotype:	IgG1, kappa
	Antigen MW:	90 kDa
Purification:	Affinity chromatography	
Conjugation:	Anti-CD19/RPE (R-phycoerythrin)	
Molar ratio	RPE/Protein is approximately 1	
Fluorescence:	RPE (Orange-Red)	Excites at 488 nm, emits at 570 nm.
Amount per vial:	100 tests (10 µL antibody to 10^6 cells).	

Reagents are not considered sterile.

STORAGE

Store in the dark at 2-8 ℃. Do not freeze. Do not u se after expiration date stamped on vial.

Alteration in the appearance of the reagent, such as precipitation, indicates instability or deterioration. In such cases, the reagent is not to be used.

PRECAUTIONS

- For *In Vitro* diagnostic use.
- Wear disposable gloves when handling any human material.
- Specimens, before and after fixation, and all material exposed to them, should be handled as if capable of transmitting infection and disposed of with proper precautions.
- This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
- Never pipette reagents by mouth and avoid their contact with skin and mucous membranes.
- Avoid microbial contamination of reagents or incorrect results may occur.
- Incubation times or temperatures other than those specified may give erroneous results; any such change must be validated by the user.
- Do not use reagents beyond expiration date for prescribed storage method. If reagents are stored under any conditions other than those specified in the specification sheet, the conditions must be validated by the user.
- Paraformaldehyde gives off formaldehyde gas when heated. Formaldehyde is toxic, allergenic, and is known to cause cancer. Use only in well-ventilated areas or in a fume hood. Avoid contact with eyes and skin clothing. If eye or skin contact occurs, immediately flush with water for at least 15 minutes and contact a physician if exposure symptoms develop. Inhalation or ingestion is harmful. If swallowed, induce vomiting and contact a physician immediately.
- Use prudent laboratory practices when handling reagents. This includes avoiding unnecessary contact, and using protective equipment such as chemical resistant gloves, eye protection, and lab coat.

Procedure

WHOLE BLOOD

Materials required, not provided

1. Blood collection tubes containing anticoagulant (K_3EDTA, Heparin or ACD)
2. Phosphate-buffered saline (PBS), 0.01M phosphate-buffered saline, pH 7.4

   Stock Solution (10x concentrate)

   80.0 g NaCl
   2.0 g KH_2PO_4
   21.6 g $Na_2HPO_4 \cdot 7H_2O$
   2.0 g KCl

   Add distilled water to 1 liter. Filter through a 0.2 µm filter

   Working Solution

   Add 100 mL of Stock Solution to 900 mL of distilled water. Store at 18-22 ℃. Filter through a 0.2 µm f ilter. Stable for up to 2 months

   (*Note:* Dako's modified Phosphate Buffered Saline (PBS) without calcium and magnesium (Dako Code S3024) can be substituted for PBS working solution)
3. 1% paraformaldehyde in PBS

   Add 1 g paraformaldehyde (Sigma-Aldrich catalog no. P6148) to 90 mL of distilled water at 70 ℃ and mi x for 15-30 minutes in a fume hood. Add 10 mL PBS Stock Solution and cool down. Filter through a 0.2 µm filter. Store at 2-8 ℃. Stable for up to three weeks
4. Erythrocyte Lytic Reagent (when using commercial reagents, follow the manufacturer's recommended protocol).

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5. Transfer pipettes (use pipettes that have non-sticking surfaces)
6. 5 mL disposable polystyrene test tubes
7. Vortex mixer
8. Centrifuge
9. Flow cytometer
10. Cell counter
11. Flow cytometer gating control such as CD45/FITC+CD14/RPE (Dako Code FR700)
12. Isotypic Control for Anti-CD19/RPE, HD37 (Dako Code X0928, IgG1/RPE)

SPECIMEN COLLECTION AND HANDLING

Collect blood specimens by venipuncture (14) into evacuated tubes containing an appropriate anticoagulant, completely expending the vacuum in the tubes. Mix the blood well with the anticoagulant to prevent clotting. At least 100 µL of whole blood is required for each test. Ideally, blood samples should be processed immediately after collection. When this is not possible, each laboratory should validate that its collection and holding methods maintain specimen integrity comparable to freshly processed material.

When using ACD or heparin anticoagulants, blood samples may be stored at 18-22 ℃ before testing for up to 3 0 hours after venipuncture. When using EDTA, blood samples should be processed within 24 hours (15, 16).

SAMPLE PERARATION

Optimum concentration of white blood cells for each test is (1 ± 0.3) x 10^7 cells/mL. For samples with a high white blood cell count, dilute samples with autologous plasma to obtain a concentration of cells approximately equal to 1 x 10^7 cells/mL.

For samples containing a low white blood cell count (less than 0.7 x 10^7 cells/mL), concentrate cells by centrifuging blood at 18-22℃ for 5 minutes at 500 x g. Draw off buffy coat with some red cells and plasma to ensure that all white blood cells are collected. Resuspend cells in autologous plasma and adjust white blood cell concentration to approximately 1 x 10^7 cells/mL. Proceed with staining procedure.

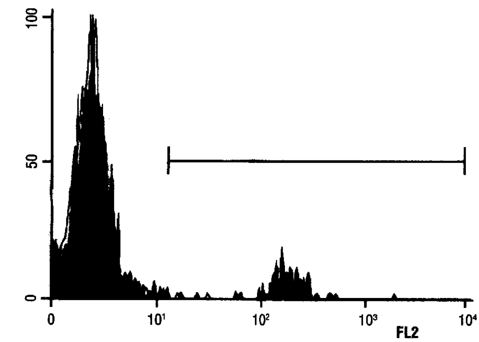
STAINING PROCEDURE

1. Use peripheral blood containing WBC's of optimal concentration.
2. Transfer 100 µL of the anticoagulated blood to the bottom of a polystyrene test tube. Any blood along the sides or top of the test tube must be removed as nonlysed RBCs and entrapped unstained WBC's may contaminate the final sample and give erroneous results.
3. Add 10 µL of Anti-CD19/RPE (Dako Code R0808). Mix by gentle tapping.
4. Two additional aliquoted samples are needed for controls: One for the isotype control IgG1/RPE (Dako Code X0928) and one for the gating control, CD45/CD14 (Dako Code FR700). Use steps 1-3 to prepare the two control samples.
5. Incubate in the dark at room temperature (18-22 ℃) for 15-30 minutes.
6. Perform erythrocyte lysing procedure. Follow the reagent manufacturer's recommendations for time and temperature of incubation. This procedure may not require a wash step and may include a fixation step, e.g. Utilyse (Dako Code S3325 or S3350).
7. If a wash step is required, cells may be centrifuged at 300 x g for 5 minutes at 18-22 ℃. Aspirate the supernatant leaving approximately 50 µL of fluid. Add 3 mL PBS. Vortex gently. Centrifuge at 300 x g for 5 minutes at 18-22 ℃, th en aspirate the supernatant, leaving approximately 50 µL of fluid. Resuspend in ~0.5 mL PBS.
8. If a fixation step is not included with the erythrocyte lysing procedure, resuspend pellet in 0.5 mL of 0.05-1% paraformaldehyde (fixative) in PBS and incubate tubes for 30 minutes (17).
9. Analyze samples on a flow cytometer within 48 hours. If lysed and fixed samples are held more than 2 hours, store at 4 ℃ in the dark until analysis (1 8, 19).

FLOW CYTOMETRIC ANALYSIS

1. Analyze cells on a flow cytometer properly standardized and gated on lymphocytes according to the instrument's operating manual.
2. Verify gate purity with the gating control tube (CD45/CD14).
3. Set the cursor using the isotype control so that 98±1% events are in the negative cell population.

REPRESENTATIVE HISTOGRAM



Lymphocytes from peripheral blood (healthy individual) labelled with RPE-Conjugated Anti-CD19, HD37 (Dako Code R0808). (13% labeled lymphocytes). Clear curve indicates negative control, IgG1/RPE, Dako Code X0928.

EXPECTED VALUES

Blood samples were collected from 150 apparently healthy males and females at three geographically separate locations. The population included adults from a variety of races ranging in age from 19 to 62 years. Samples were stained with Anti-CD19/RPE, HD37, monoclonal antibody. Normal CD19 positive B cell values were measured by flow cytometry using the whole blood method, and are presented in the following table. Values are expressed as a percent of the total lymphocyte count and **are intended as representative values only. Each laboratory should establish its own expected values from the local population of normal donors.**

		95% Range		
	N	**Min**	**Max**	**Mean ± 1 SD**
%B Lymphocytes	150	4.47	25.60	12.14 ± 5.38

PHERIPHERAL BLOOD MONONUCLEAR CELLS (SEPARATED CELLS)

Materials Required, Not Provided

1. Blood collection tubes containing anticoagulant (K_3EDTA, Heparin or ACD).
2. Phosphate-buffered saline (PBS), 0.01M Phosphate-Buffered Saline, pH 7.4.

   Stock Solution (10x concentrate)

   80.0 g NaCl
   2.0 g KH_2PO_4
   21.6 g $Na_2HPO_4 \cdot 7H_2O$
   2.0 g KCl

   Add distilled water to 1 liter. Filter through a 0.2 µm filter.

   Working Solution

   Add 100 mL of Stock Solution to 900 mL of distilled water. Store at 18-22 ℃. Filter through a 0.2 µm f ilter. Stable for up to 2 months.

   (*Note:* Dako's modified Phosphate Buffered Saline (PBS) without calcium and magnesium (Dako Code S3024) can be substituted for PBS working solution)
3. 1% paraformaldehyde in PBS.

   Add 1 g paraformaldehyde (Sigma-Aldrich catalog no. P6148) to 90 mL of distilled water at 70 ℃ and mi x for 15-30 minutes in a fume hood. Add 10 mL PBS Stock Solution and cool down. Filter through a 0.2 µm filter. Store at 4 ℃. Stable for up to three weeks.
4. Ficoll containing reagent
5. Transfer pipettes (use pipettes that have non-sticking surfaces)

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6. 5 mL disposable polystyrene test tubes
7. Vortex mixer
8. Centrifuge
9. Flow cytometer
10. Cell counter
11. Flow cytometer gating control such as CD45/FITC+CD14/RPE (Dako Code FR700).
12. Isotypic Control for Anti-CD19/RPE (Dako Code X0928, IgG1/RPE)
13. 15 mL centrifuge tubes (tissue culture plasticware or siliconized glassware)
14. Isotonic medium (e.g. PBS with 1% w/v BSA and 0.015 mol/L NaN_3)
15. Trypan Blue (Sigma-Aldrich catalog No. T-6146)

SPECIMEN PREPARATION

(See Product Specification Sheet for Ficoll-Paque®)

1. Carefully layer 8 mL of diluted blood (dilute 1 to 1 with isotonic medium) on top of 4 mL of Ficoll-Paque in a 15 mL centrifuge tube.
2. Centrifuge at 18-22 ℃ at 400 x g for 30 minute s. Mononuclear cells should form a visible, clean interface between the plasma and Ficoll-Paque.
3. Collect the layer of cells by inserting a thin pipette through the plasma. Place collected cells in a 15 mL test tube containing 5 mL isotonic medium. Spin at 200 x g for 10 minutes. Aspirate and discard the supernatant.
4. Wash once more with the isotonic medium and centrifuge at 200 x g for 10 minutes.
5. Resuspend cells in the isotonic medium. The cells can then be counted and evaluated for cell viability and further studies.

CELL VIABILITY

1. Dissolve Trypan Blue in PBS to a final concentration of 5 g/L.
2. Mix equal volumes of cell suspension and Trypan Blue solution.
3. Place a sample of the mixture in a hemocytometer chamber. Do not overfill or underfill the chamber.
4. Count the live and dead (blue) cells. Calculate the percentage of viable cells from the number of live and dead cells.

(Note: The cell count should be performed between 5 and 15 minutes after addition of the dye. Trypan Blue has a greater affinity for serum proteins than for cellular proteins. If the background is too dark, cells should be washed in protein-free medium by centrifugation.)

STAINING PROCEDURE

1. Obtain a washed suspension of isolated mononuclear cells and adjust cell concentration to approximately 1 x 10^7 per mL.
2. Place approximately 1 x 10^6 cells/100 µL into the bottom of a polystyrene test tube.
3. Add 10 µL of Anti-CD19/RPE, HD37, antibody (Dako Code R0808). Mix by gentle tapping.
4. Two additional aliquoted samples are needed for control: One for the isotype control, IgG1/RPE (Dako Code X0928) and one for the gating control (Dako Code FR700, CD45/CD14). Use steps 1-3 to prepare the two control samples.
5. Incubate in the dark at 4 ℃ for 30 minutes.
6. Wash in approximately 2 mL of PBS. Centrifuge at 200 x g for 5 minutes at 18-22℃. Aspirate the sup ernatant.
7. Resuspend pellet in 0.3 mL of 1% paraformaldehyde (fixative) in PBS.
8. Analyze on a flow cytometer within 48 hours.

FLOW CYTOMETRIC ANALYSIS

1. Analyze cells on a flow cytometer properly standardized and gated on lymphocytes according to the instrument operating manual.
2. Verify gate purity with the control tube (CD45/CD14).
3. Set the cursor using the isotypic control so that 98 ± 1% events are in the negative cell population.

EXPECTED VALUES

Blood samples were collected from 150 apparently healthy males and females at three geographically separate locations. The population included adults from a variety of races ranging in age from 19 to 62 years. Samples were stained with Anti-CD19/RPE, HD37, monoclonal antibody. Normal CD19 positive B lymphocyte values were measured by flow cytometry using the PBMC method, and are presented in the following table. Values are expressed as % of the total lymphocyte count and **are intended as representative values only. Each laboratory should establish its own expected values from the local population of normal donors.**

	n	95% Range Min	Max	Mean ± 1 SD
%B lymphocytes	150	2.47	24.27	9.10 ± 5.40

QUALITY ASSURANCE

Peripheral blood from a normal, apparently healthy donor should be run as a positive control to ensure proper working conditions. Normal ranges should be established within a local population of normal, apparently healthy donors.

Interference in the counts of percent positive cells by monocytes and granulocytes with Fc receptor bound antibody may be reduced by proper flow cytometric gating on lymphocytes.

An appropriate isotype negative control that is non-human reactive (IgG1/RPE, Dako Code X0928) is used to control for nonspecific antibody binding to lymphocytes in each patient sample.

The brightly stained lymphocyte population is measured within markers set to exclude the low level of nonspecific fluorescence. The brightly-stained lymphocyte population is measured within markers set to exclude the low level of nonspecific fluorescence.

Nonspecific fluorescence above the background cursor is usually limited to 1-2% in normal individuals. If the background level above the cursor for any control sample is greater than 1-2%, the test results may be erroneous.

LIMITATIONS

Blood samples should be stained within 30 hours of collection for optimal results. Retain samples in the original sample tube at room temperature prior to staining and analysis. Do not refrigerate. Refrigerated samples or samples stored longer than 30 hours may give erroneous results. To ensure maximum validity, analyze stained cells promptly.

Samples from certain patients may present special problems due to abnormal erythrocyte or lymphocyte populations that are a result of illness or drug usage.

Blood samples from abnormal donors may not show abnormal values for the percent of lymphocytes stained with a monoclonal antibody. Results obtained from flow cytometric analysis should be reviewed with results from other diagnostic procedures.

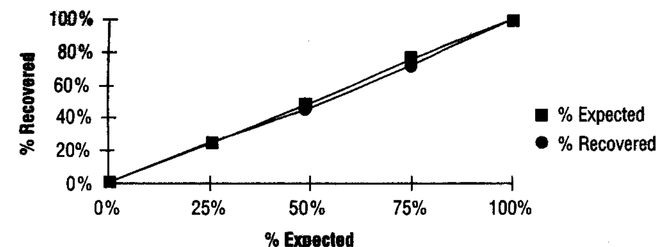
Accurate values obtained by flow cytometric procedures depend upon correct alignment and calibration of the laser, as well as proper gating and compensation processes.

Peripheral blood mononuclear cells (PBMCs) obtained by means of density gradient separation may not have the same relative percentages of lymphocytes as whole blood preparations. For normal patients, this factor may not be significant. For patients with leukopenia, the selective exclusion of specific lymphocyte subsets may affect the accuracy of the measurement.

LINEARITY

A test of the Anti-CD19/RPE, HD37, monoclonal antibody was performed to determine the linearity of the binding to the CD19 cluster determinant. Raji cell line suspended cells (positive epitope for the CD19 antibody) were mixed with JM cell line suspended cells (negative for the CD19 antibody) at several concentrations to test for binding linearity. Anti-CD19/RPE, HD37, bound with the Raji cells on a linear basis ($y = -0.49 + 0.99\ x$, $r^2 = 1.0$) with the slope approaching 1.0.

Anti-CD19/RPE (R0808) LINEARITY



REPRODUCIBILITY

Ten replicate measurements of each of three levels (normal, high and low) of CD19+ lymphocyte levels were analyzed on a Becton-Dickinson FACScan® and Coulter Profile II flow cytometers. High levels of CD19+ lymphocytes were achieved by depleting the normal peripheral blood of CD3+ lymphocytes. Low levels were achieved by depletion of CD19+ lymphocytes. Depletion of CD3+ or CD19 positive cells was achieved by incubating normal peripheral blood with beads to which the depleting antibody had been attached. Values are expressed as a percent of the total lymphocyte count.

FACScan	Level	Mean % CD19+	±1 SD	% CV
	1	79.9	0.5	0.6
	2	13.4	1.0	7.5
	3	2.3	0.2	8.7
Profile II		**Mean % CD19+**	**±1 SD**	**% CV**
	1	77.8	0.9	1.2
	2	13.3	1.0	7.5
	3	2.8	0.5	17.9

SPECIFICITY

Background (non-specific staining) can be subtracted from flow cytometry measurements by using test samples stained with appropriate mouse isotypic control antibodies. The positive stained population can be set to exclude this low level of fluorescence. Antibody binding to non-lymphocytic cells may be excluded by proper gating on the flow cytometer.

Specificity of Anti-CD19/RPE, HD37, has been verified by tests performed on five apparently healthy adult donors of various races at Dako. Cell populations tested were RBCs, granulocytes, monocytes, lymphocytes and platelets. The results indicate antibody binding of Anti-CD19/RPE, HD37, is specific for lymphocytes.

Anti-CD19/RPE, HC37 Specificity

	% Positive Red Blood Cells	% Positive Granulocytes	% Positive Monocytes	% Positive Lymphocytes	% Positive Platelets
Average (n=5)	0.32	0.58	0.66	10.48	0.54
(range)	(0.1-1.0)	(0.2-1.2)	(0.0-1.5)	(5.3-18.7)	(0.2-0.8)

In addition, Anti-CD19/RPE, HD37, is unreactive with other cells in the human hematopoietic system and shows no reaction with non-hematopoietic cells, e.g. in kidney, liver, breast or lung tissues (18).

REFERENCES

1. Calvelli T, Denny TN, Paxton H, Gelman R, Kagan J. Guideline for flow cytometric immunophenotyping: a report from The National Institute of Allergy and Infectious Diseases, Division of AIDS. Cytometry 1993;14:702
2. Centers for Disease Control. Guidelines for the performance of CD4+ T-cell determinations in persons with human immunodeficiency virus (HIV). MMWR 1997;46 (RR-2):1
3. National Committee for Clinical Laboratory Standards (1998): Clinical applications of flow cytometry: Quality assurance and immunophenotyping of lymphocytes; Approved Guideline. NCCLS Document H42-A (ISBN 1-56238-364-7). NCCLS, Villanova, PA, Vol. 18, No. 21
4. Pezzutto A, Dörken B, Feller A, Moldenhauer G, Schwartz R, Wernet P, et al. HD37 monoclonal antibody: A useful reagent for further characterization of "non-T, non-B" lymphoid malignancies. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ID, eds. Leukocyte Typing II, Vol. 2. New York-Berlin-Heidelberg-Tokyo: Springer Verlag, 1986:391
5. Stamenkovic I, Seed B. CD19, the earliest differentiation antigen of the B cell-lineage, bears three extracellular immunoglobulin-like domains and an Epstein-Barr virus-related cytoplasmic tail. J Exp Med 1988;168:1205
6. Nadler LM. B cell leukemia panel workshop: Summary and comments. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ID, eds. Leukocyte Typing II, Vol. 2. New York-Berlin-Heidelberg-Tokyo: Springer Verlag, 1986:3
7. Loken MR, Shah VO, Hollander Z, Civin CI. Flow cytometric analysis of normal B lymphoid development. Pathol Immunopathol Res 1988; 7:357
8. Ledbetter JA, Rabinovitch PS, June CH, Song CW, Clark EA, Uckun FM. Antigen-independent regulation of cytoplasmic calcium in B cells with a 12-kDa B-cell growth factor and anti-CD19. Proc Natl Acad Sci (USA) 1988;85:1897
9. DeRie MA, Schumacher TNM, Van Schijndel GMW, Van Lier RAW, Miedema F. Regulatory role of CD19 molecules in B-cell activation and differentiation. Cell Immunol 1989;118:368
10. Uckun FM, Jaszcz W, Ambrus JL, Fanci AS, Gajl-Peczalska K, Song CW, et al. Detailed studies on expression and function of CD19 surface determinant by using B43 monoclonal antibody and the clinical potential of anti-CD19 immunotoxins. Blood 1988;71:13
11. Clark EA, Einfeld D. Human B cell surface molecules defined by an international workshop panel of monoclonal antibodies. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ID, eds. Leukocyte Typing II, Vol. 2. New York-Berlin-Heidelberg-Tokyo: Springer Verlag, 1986:155
12. Schmidt RE. Monoclonal antibodies for diagnosis of immunodeficiencies. Blut 1989;59:200
13. de Martini RM, Parker JW. Immunologic alterations in human immunodeficiency virus infection: a review. J Clin Lab Anal 1989;3:56
14. National Committee for Clinical Laboratory Standards (1997): Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue; Approved Guideline. NCCLS Document M29-A (ISBN 1-56238-339-6). NCCLS, Villanova, PA
15. Nicholson JK, Green TA. Selection of anticoagulants for lymphocyte immunophenotyping. Effect of specimen age on results. J Immunol Methods 1993;165:31
16. Paxton H, Bendele T. Effect of time, temperature, and anticoagulant on flow cytometry and hematological values. Ann NY Acad Sci 1993;677:440
17. Koepke JA, and Landay AL. Precision and accuracy of absolute lymphocyte counts. Clin Immuno and Immunopath 1989;52:19
18. Mason DY, Ladyman H, Gatter KC. Immunohistochemical analysis of monoclonal anti-B cell antibodies. In: Reinherz, EL, Haynes, BF, Nadler, LM, Bernstein, ID, eds. Leukocyte Typing II, Vol. 2. New York-Berlin-Heidelberg-Tokyo: Springer Verlag, 1986:245
19. Lewis DE. Cytochemistry I: Cell surface immunofluorescence. In: Bauer KD, Duque RE, Shankey TV, editors. Clinical flow cytometry. Willliams & Wilkins, Baltimore, MD; 1993. p. 144

Explanation of symbols

REF	Catalogue number	2°C–8°C	Temperature limitation		Use by
IVD	In vitro diagnostic medical device		Keep away from sunlight (consult storage section)		Manufacturer
	Consult instructions for use	LOT	Batch code		

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