Monoclonal Antibody to CD19, PE conjugated (CD19 PE)

Cat.No. ED7017

1. Intended purpose

The reagent CD19 PE permits identification and enumeration of cell populations expressing human CD19 antigen in whole blood using flow cytometry.

2. Test principle

This test is based on specific binding of monoclonal antibody to the antigenic determinant expressed on the surface of leukocytes. The monoclonal antibody is labeled with fluorochrome which is excited via laser beam from a flow cytometer during analysis. Subsequent emission of light from fluorochromes of each cell is collected and analyzed by a flow cytometer. The fluorescence intensity differences enable separation of cell subsets based on expression of analyzed antigen.
Specific staining of blood cells is performed by incubation of blood samples with the reagent followed by a lysis of red blood cells. Afterwards, unaffected leukocytes are subjected to analysis by a flow cytometer.

3. Reagents provided

The reagent contains mouse monoclonal antibody against human CD19 antigen (clone LT19) which was purified by affinity chromatography and labeled with R-Phycoerythrin (PE). The labeled antibody is diluted at optimum concentration in stabilizing phosphate buffered saline (PBS) solution containing 15mM sodium azide. The content of a vial (2 ml) is sufficient for 100 tests.

Product specification

Content	100 tests, 2 ml
Usage	20 µl per test
Specificity	Human CD19
Clone	LT19
Isotype	Mouse IgG1
Fluorochrome	PE
λ excitation	488 nm
Emission maximum	575 nm

4. Materials required but not provided

Test tubes for blood staining (e.g. 12 × 75 mm)
Commercial lysing solution
Phosphate buffered saline (PBS)
Isotype control antibody (mouse IgG1 PE)

5. Equipment required

Automatic pipettes with disposable tips
Vortex mixer
Centrifuge
Flow cytometer with excitation laser 488 nm and proper filters

6. Storage and handling

Store the vial at 2 - 8 °C. Keep away from sunlight. Do not freeze. Do not aliquot. Expiration date is stated on a vial label and on outer packaging.

7. Warnings, precautions and limitations of use

- Intended for In Vitro Diagnostic use in laboratories outside USA and Canada. This reagent is in conformity with the European Directive 98/79/EC.
- Do not use reagent after expiration date.
- Avoid reagents contamination.
- Avoid prolonged exposure to light.
- The content of the vial must not freeze.
- Any non-performance of staining protocol may produce false results.
- The reagent contains sodium azide (NaN_3) which is highly toxic in pure form. However, the concentration in the reagent (15mM) is not considered as hazardous. When disposing the reagent, flush the sink with a large volume of water.
- Blood samples are considered as potentially infectious and must be handled with care. Avoid all contact of the sample with the skin, eyes and mucosa.
- In case of hyperleukocytose sample, it is recommended to dilute blood sample with PBS to obtain leukocyte density approximately 5×10^6 leukocytes/ml.
- Blood samples from abnormal patients may exhibit abnormal values of positive cells.
- Data may be incorrectly interpreted if fluorescent signals were compensated wrongly or if gates were positioned inaccurately.
- Flow cytometer may produce false results if the device has not been aligned and maintained appropriately.
- Red blood cells from abnormal patients may be resistant to lysis using lysing solutions.
- Blood samples should be stained and analyzed within 24 hours from the blood collection.

8. Specimen

Use the peripheral human blood in a sterile tube with an anticoagulant (Heparin or EDTA).
Blood must be stored at room temperature.

9. Procedure

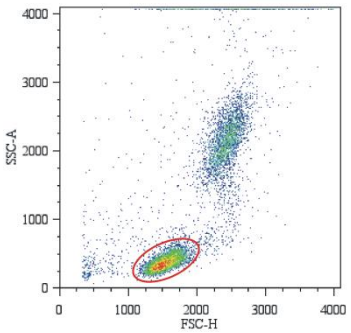
Staining protocol

1. Add 20 µl of CD19 PE reagent to a test tube, and the necessary amount of isotype control to a control tube.
2. Add 100 µl of blood sample to each tube. Vortex the tubes.
3. Incubate tubes for 20 - 30 minutes at room temperature in the dark.
4. Perform lysis of red cells using lysing solution. It is recommended to use a commercial lysing solution containing formaldehyde as a fixative. Follow the instructions of the lysing solution manufacturer.
5. Centrifuge tubes for 5 minutes at 300 g.
6. Remove supernatant and resuspend pellet with 3 - 4 ml of PBS.
7. Centrifuge tubes for 5 minutes at 300 g.
8. Remove supernatant and resuspend pellet with 0.3 – 0.5 ml of PBS.
9. Analyze samples immediately using flow cytometer or store samples at 2 - 8 °C in the dark and analyze within 24 hours provided that cells were fixed.

Flow Cytometric Analysis

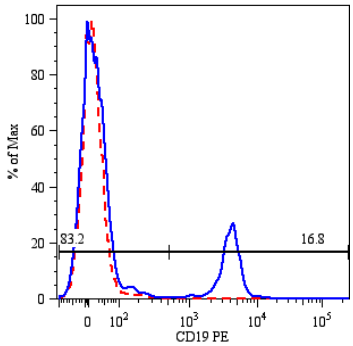
Analyze sample stained with CD19 PE using a flow cytometer. Visualize recorded data on the side-scatter (SSC) versus forward-scatter (FSC) plot. Set the gate for lymphocyte population as shown in figure 1.

Fig. 1: Delimitation of lymphocyte population



Then make a histogram of lymphocytes with PE intensity on the x-axis as shown in figure 2. Separate positive and negative populations using appropriate gates and calculate the percentage of CD19 positive lymphocytes. The region corresponding to the negative population should be set up using control cells which were stained by isotype control antibody.

Fig. 2: Lymphocytes stained with CD19 PE reagent



10. Analytical performance

Specificity

The antibody LT19 reacts with CD19 (B4), a 95 kDa type I transmembrane glycoprotein of immunoglobulin superfamily, expressed on B lymphocytes and follicular dendritic cells; lost on plasma cells.

11. Clinical performance

Expected values

Results obtained in different laboratories may vary. Each laboratory should establish a normal range of cell subsets using its own test conditions. In our laboratory, the reagent CD19 PE was tested on 40 blood samples of healthy people. Obtained results are given in the table below.

Parameter	Mean (%)	SD	CV (%)
$CD19^+$ lymphocytes	12.6	5.0	39.7

12. References

Shi X et al. (2007) CD19 hyperexpression augments Sle1-induced humoral autoimmunity but not clinical nephritis. Arthritis Rheum. 56: 3057-3069

van Zelm MC et al. (2006) An antibody-deficiency syndrome due to mutations in the CD19 gene. N Engl J Med. 354: 1901-1912

Lin CW et al. (2005) CD94 1A transcripts characterize lymphoblastic lymphoma/leukemia of immature natural killer cell origin with distinct clinical features. Blood. 106: 3567-74

Elias F et al. (2003) Strong cytosine-guanosine-independent immunostimulation in humans and other primates by synthetic oligodeoxynucleotides with PyNTTTTGT motifs. J Immunol. 171: 3697-704

Inabe K and Kurosaki T (2002) Tyrosine phosphorylation of B-cell adaptor for phosphoinositide 3-kinase is required for Akt activation in response to CD19 engagement. Blood 99: 584-589

Fujimoto M et al. (1999) CD19 amplifies B lymphocyte signal transduction by regulating Src-Family protein tyrosine kinase activation. J Immunol. 162: 7088-7094

13. Manufacturer

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14. Trademarks

N/A

15. Revision History

• Version 1, ED7017_IFU_v1
Initial Release
• Version 2, ED7017_IFU_v2
Merging three language mutations into one document.
• Version 3, ED7017_IFU_v3
The address was changed: “Nad Safinou II 341”.
• Version 4, ED7017_IFU_v4
Precautions section was changed – “Intended for professional use only.” – removed. “Intended for In Vitro Diagnostic use in laboratories outside USA and Canada. This CE-IVD reagent is in conformity with the European In Vitro Diagnostic Medical Device Directive 98/79/EC.” – added.
• Version 5, ED7017_IFU_v5
Reagent provided section was changed: text “stabilizing” added, “solution” – added and “0.2% (w/v) high-grade protease free Bovine Serum Albumin (BSA) as a stabilizing agent” – removed.
• Version 6, ED7017_IFU_v6
The company logo changed. IFU layout changed. “Keep away from sunlight.” – added. Postal code changed:” 25250 Vestec”.

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100 tests | Cat.No. **ED7017**

IVD CE

Instructions for Use

Version: ED7017_IFU_v6_EN
Date of Issue: 30-07-2020

EN

Symbols

REF	Catalogue number
LOT	Batch code
	Use-by date
	Temperature limits
	Keep away from sunlight
IVD	In vitro diagnostic medical device
CE	CE marking of conformity
	Consult instructions for use
	Manufacturer