

Cat. No. ED7017

Monoclonal Antibody to CD19, PE conjugated (CD19 PE)

1. Specification

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

2. Intended use

The reagent CD19 PE permits identification and enumeration of cell populations expressing human CD19 antigen in whole blood using flow cytometry. The reagent could be used in various antibody panels for multi-parameter flow cytometry analyses.

3. 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.

4. 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.

5. Reagent 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 in an optimal concentration in phosphate buffered saline (PBS) containing 15mM sodium azide and 0.2% (w/v) high-grade protease free Bovine Serum

Albumin (BSA) as a stabilizing agent. The content of a vial (2 ml) is sufficient for 100 tests.

6. Storage

Store vial in the dark at 2-8°C. Do not freeze.

7. Precautions

- Intended for professional use only.
- Do not use after expiration date stamped on vial label.
- Avoid prolonged exposure to light.
- The content of the vial must not freeze.
- Avoid contamination of the reagent.
- Any non-performance of staining protocol may produce false results.
- The reagent contains sodium azide (NaN₃) 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 large volume of water to avoid accumulation of explosive metal-azide in plumbing.
- 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.

8. Necessary material not supplied

Material necessary for collection of peripheral blood, test tubes for staining of blood samples (e.g. 12 × 75 mm), automatic pipettes with disposable tips, vortex mixer, centrifuge, commercial lysing solution, phosphate buffered saline (PBS), isotype control antibody (mouse IgG1 PE), flow cytometer.

9. Staining protocol

1. Collect peripheral blood in a sterile tube with an anticoagulant (e.g. Heparin, EDTA).
2. Add 20 μ l of CD19 PE reagent to a test tube, and the necessary amount of isotype control to a control tube.

3. Add 100 μ l of blood sample to each tube. Vortex the tubes.
4. Incubate tubes for 20-30 minutes at room temperature in the dark.
5. Perform lysis of red cells using lysing solution. It is recommended to use a commercial lysing solution containing paraformaldehyde as a fixative (e.g. ADG-LYSE produced by AN DER GRUB, Cat. No. GAS-003). Follow the instructions of the lysing solution manufacturer.
6. Centrifuge tubes for 5 minutes at 300 g.
7. Remove supernatant and resuspend pellet with 3-4 ml of PBS.
8. Centrifuge tubes for 5 minutes at 300 g.
9. Remove supernatant and resuspend pellet with 0.3 – 0.5 ml of PBS.
10. 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.

10. Data 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 on figure 1. Then make a histogram of lymphocytes with PE intensity on the x-axis as shown on 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.

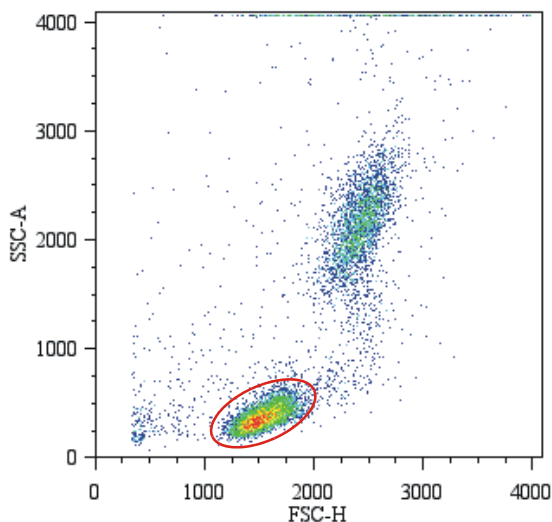


Fig. 1: Delimitation of lymphocyte population

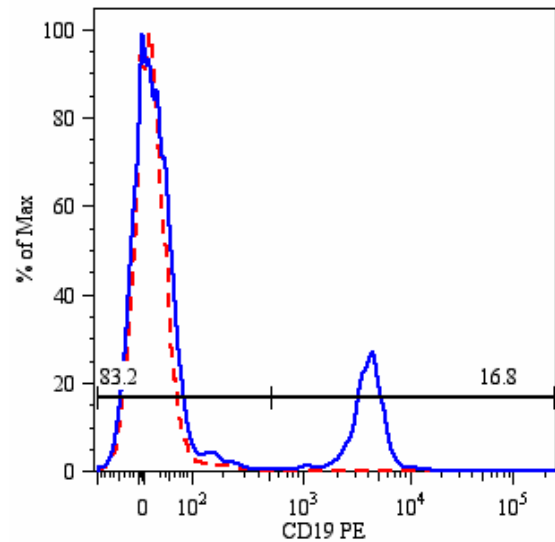


Fig. 2: Lymphocytes stained with CD19 PE reagent

11. 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. Limitations

- Flow cytometer may produce false results if the device has not been aligned and maintained appropriately.
- Data may be incorrectly interpreted if fluorescent signals were compensated wrongly or if gates were positioned inaccurately.
- Blood samples from abnormal patients may exhibit abnormal values of positive cells.
- Red blood cells from abnormal patients may be resistant to lysis using lysing solutions.
- 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 should be stained and analyzed within 24 hours from the blood collection.

13. References

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

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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









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

14. Manufacturer

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15. Explanation of symbols

| | | | |
|---|------------------------------------|---|---------------------------------|
|  IVD | In Vitro Diagnostic Medical Device |  | Sufficient for N test |
|  REF | Catalog number |  | Store within temperature limits |
|  | Manufacturer identification |  LOT | Batch code |
|  | Consult the manual before use |  | Use by |