Monoclonal Antibody to CD16, PE conjugated (CD16 PE)

Cat.No. ED7016

1. Intended purpose

The reagent CD16 PE permits identification and enumeration of cell populations expressing human CD16 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 CD16 antigen (clone LNK16) 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 CD16
Clone	LNK16
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 aliquote. 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 CE-IVD kit 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

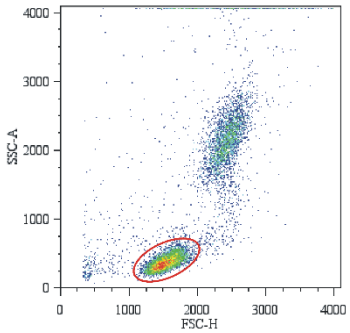
Staining protocol

1. Add 20 µl of CD16 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

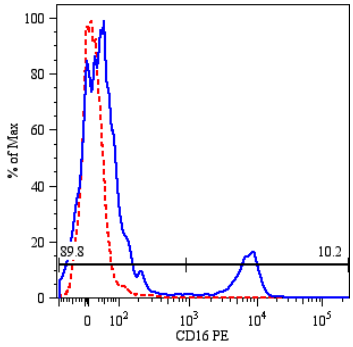
Analyze sample stained with CD16 PE using a flow cytometer. Visualize recorded data on the side-scatter (SSC) versus forward-scatter (FSC) plot. Set the gate for lymphocyte population as shown in figure 1.

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 CD16 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 CD16 PE reagent.



10. Analytical performance

Specificity

The antibody LNK16 reacts with CD16, a low affinity receptor for aggregated IgG (FcγRIII antigen). CD16 exists in two different isoforms: CD16a (FcγRIIIA; 50-65 kDa; expressed on NK-cells, monocytes and macrophages) and CD16b (FcγRIIIB; 48 kDa; mainly expressed on neutrophils). The monoclonal antibody LNK16 was assigned to CD16 during the Human Leukocyte Differentiation Antigen workshop (*HLDA5 WS Code: M MA069, NK NK50*).

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 CD16 PE was tested on 40 blood samples of healthy people. Obtained results are given in the table below.

Parameter	Mean (%)	SD	CV (%)
CD16+ lymphocytes	11.3	5.0	44.3

12. References

Boyle JJ (2004) Human macrophages kill human mesangial cells by Fas-L-induced apoptosis when triggered by antibody via CD16. Clin Exp Immunol. 137: 529-37

Arase N et al. (2003) IgE-mediated activation of NK cells through Fc gamma RIII. J Immunol. 170: 3054-8

Kocher M et al. (1997) Cross-linking of Fc gamma receptor IIa and Fc gamma receptor IIIb induces different proadhesive phenotypes on human neutrophils. J Immunol. 159: 3940-8

Tamm A and Schmidt RE (1996) The binding epitopes of human CD16 (Fc gamma RIII) monoclonal antibodies. Implications for ligand binding. J Immunol. 157: 1576-81

Gessner JE et al. (1995) The human low affinity immunoglobulin G Fc receptor III-A and III-B genes. Molecular characterization of the promoter regions. J Biol Chem. 270: 1350-61

Leukocyte Typing V., Schlossman S. et al. (Eds.), Oxford University Press (1995).

13. Manufacturer

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14. Trademarks

N/A

15. Revision History

• Version 1, ED7016_IFU_v1
Initial Release
• Version 2, ED7016_IFU_v2
The address was changed: "Nad Safinou II 341"
"The reagent could be used in various antibody panels for multi-parameter flow cytometry analyses." – removed.
• Version 3, ED7016_IFU_v3
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 4, ED7016_IFU_v4
Precautions section was changed – stabilizing added, solution – added and "0.2% (w/v) high-grade protease free Bovine Serum Albumin (BSA) as a stabilizing agent" – removed.
• Version 5, ED7016_IFU_v5
The company logo changed. IFU layout changed. "Keep away from sunlight." – added. "Blood must be stored at room temperature." - added. Postal code changed – "25250 Vestec"

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100 tests | Cat.No. ED7016

IVD CE

Instructions for Use

Version: ED7016_IFU_v5_EN
Date of Issue: 27-02-2020

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