Monoclonal Antibody to CD3, PE conjugated (CD3 PE)

Cat.No. ED7002

1. Intended purpose

The reagent CD3 PE permits identification and enumeration of cell populations expressing human CD3 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 CD3 antigen (clone MEM-57) which was purified by affinity chromatography and labeled with R-Phycoerythrin (PE). The labeled antibody is diluted in an optimal 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 CD3
Clone	MEM-57
Isotype	Mouse IgG2a
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 IgG2a 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

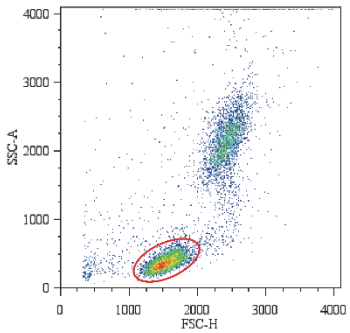
Staining protocol

1. Add 20 µl of CD3 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

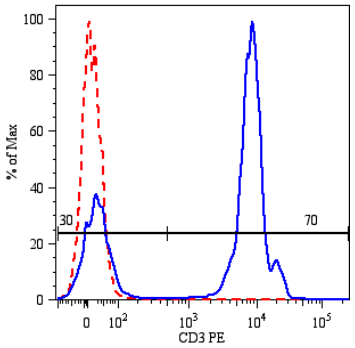
Analyze the sample stained with CD3 PE using a flow cytometer. Visualize recorded data on the side-scatter (SSC) versus forward-scatter (FSC) plot. Set the gate for lymphocyte population as shown in figure 1.

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 CD3 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 CD3 PE reagent



10. Analytical performance

Specificity

The monoclonal antibody MEM-57 reacts with gamma-epsilon and delta-epsilon dimers of human CD3 complex, a part of a bigger multisubunit T cell receptor complex (CD3/TCR) expressed on peripheral blood T lymphocytes and mature thymocytes.
HLDA4 WS Code: T 96

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. Results obtained in our laboratory are given in the table below.

Parameter	Mean (%)	SD	CV (%)
$CD3^+$ lymphocytes	72.4	7.2	9.9

12. References

Alarcon B et al. (2006) T-cell antigen-receptor stoichiometry: pre-clustering for sensitivity. EMBO Rep. 7: 490-5

Kuhns MS et al. (2006) Deconstructing the form and function of the TCR/CD3 complex. Immunity 24:133-9

Huang Y and Wange RL (2004) T cell receptor signaling: beyond complex complexes. J Biol Chem. 279: 28827-28830

Hilgert I. et al. (1993) Therapeutic in vivo use of the A1-CD3 monoclonal antibody. Transplantation 55: 435

Horejsi V. et al. (1988) Monoclonal antibodies against human leucocyte antigens. II. Antibodies against CD45 (T200), CD3 (T3), CD43, CD10 (CALLA), transferrin receptor (T9), a novel broadly expressed 18-kDa antigen (MEM-43) and a novel antigen of restricted expression (MEM-74). Folia Biol. (Praha) 34: 23

Leukocyte Typing IV., Knapp W. et al. (Eds.), Oxford University Press (1989); p. 293.

Leukocyte Typing III., McMichael M.J. et al. (Eds.), Oxford University Press (1987); p.611.

13. Manufacturer

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14. Trademarks

N/A

15. Revision History

• Version 1, ED7002_IFU_v1
Initial Release
• Version 2, ED7002_IFU_v2
Merging three language mutations into one document.
• Version 3, ED7002_IFU_v3
The address was changed: “Nad Safinou II 341”.
• Version 4, ED7002_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, ED7002_IFU_v5
Precautions 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, ED7002_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. **ED7002**

IVD CE

Instructions for Use

Version: ED7002_IFU_v6_EN
Date of Issue: 14-05-2020

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