

# Monoclonal Antibody to CD4, PE conjugated (CD4 PE)

Cat.No. ED7003

## 1. Intended purpose

The reagent CD4 PE permits identification and enumeration of cell populations expressing human CD4 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 CD4 antigen (clone MEM-241) 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 CD4
Clone	MEM-241
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 (Na<sub>2</sub>N<sub>3</sub>) 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<sup>5</sup> 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

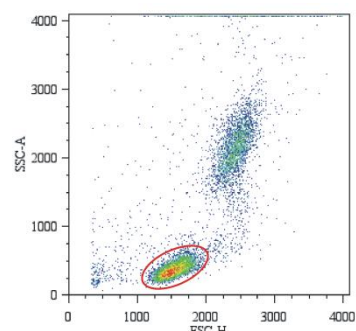
### Staining protocol

- Add 20 µl of CD4 PE reagent to a test tube, and the necessary amount of isotype control to a control tube.
- Add 100 µl of blood sample to each tube. Vortex the tubes.
- Incubate tubes for 20 - 30 minutes at room temperature in the dark.
- 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.
- Centrifuge tubes for 5 minutes at 300 g.
- Remove supernatant and resuspend pellet with 3 - 4 ml of PBS.
- Centrifuge tubes for 5 minutes at 300 g.
- Remove supernatant and resuspend pellet with 0.3 - 0.5 ml of PBS.
- 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

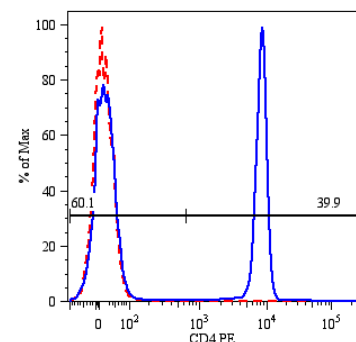
Analyze the sample stained with CD4 PE using a flow cytometer. Visualize recorded data on the side-scatter (SSC) versus forward-scatter (FSC) plot. Set the gate for lymphocyte population as shown in figure 1.

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 CD4 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 CD4 PE reagent



## 10. Analytical performance

### Specificity

The antibody MEM-241 recognizes CD4 coreceptor, a 55 kDa transmembrane glycoprotein of immunoglobulin family expressed on subsets of T lymphocytes (such as "helper" T cells, CD4<sup>+</sup> regulatory T cells or CD4<sup>+</sup>CD8<sup>+</sup> double-positive T cells) and also on monocytes, tissue macrophages and granulocytes. The monoclonal antibody MEM-241 was assigned to CD4 during the Human Leukocyte Differentiation Antigen workshop (HLDA8 (HCDM) WS Code: M241).

## 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 (%)
CD4 <sup>+</sup> lymphocytes	43.9	9.1	20.7

## 12. References

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- Huang Y and Wange RL (2004) T cell receptor signaling: beyond complex complexes. *J Biol Chem*. 279: 28827-28830
- Nakamura Y et al. (2004) Intrathyroidal CD4<sup>+</sup> T lymphocytes express high levels of Fas and CD4<sup>+</sup>CD8<sup>+</sup> macrophages/dendritic cells express Fas ligand in autoimmune thyroid disease. *Thyroid* 14: 819-824
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- Foti M et al. (2002) p56Lck anchors CD4 to distinct microdomains on microvilli. *Proc Natl Acad Sci U S A*. 99: 2008-13

Nam K-H et al. (2000) Peripheral blood extrathymic CD4<sup>+</sup>CD8<sup>+</sup> T cells with high cytotoxic activity are from the same lineage as CD4<sup>+</sup>CD8<sup>+</sup> T cells in cynomolgus monkeys. *Int Immunol*. 12: 1095-1103

Millan J et al. (1999) CD4 segregates into specific detergent-resistant T-cell membrane microdomains. *Tissue Antigens*. 53: 33-40

## 13. Manufacturer

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## 14. Trademarks

N/A

## 15. Revision History

- Version 1, ED7003\_IFU\_v1  
Initial Release
- Version 2, ED7003\_IFU\_v2  
Merging three language mutations into one document.
- Version 3, ED7003\_IFU\_v3  
The address was changed: "Nad Safinou II 341".
- Version 4, ED7003\_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, ED7003\_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, ED7003\_IFU\_v6  
The company logo changed. IFU layout changed. "Keep away from sunlight." - added. Postal code changed: "25250 Vestec"

# exbio

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100 tests | Cat.No. ED7003



### Instructions for Use

Version: ED7003\_IFU\_v6\_EN  
Date of Issue: 23-06-2020

EN

### Symbols

The REF symbol consists of the letters 'REF' in a bold, sans-serif font, enclosed within a rectangular border.	Catalogue number
The LOT symbol consists of the letters 'LOT' in a bold, sans-serif font, enclosed within a rectangular border.	Batch code
The use-by date symbol is an icon of an hourglass.	Use-by date
The temperature limits symbol is an icon of a thermometer.	Temperature limits
The keep away from sunlight symbol is an icon of a sun with rays.	Keep away from sunlight
The IVD symbol consists of the letters 'IVD' in a bold, sans-serif font, enclosed within a rectangular border.	In vitro diagnostic medical device
The CE marking symbol consists of the letters 'C' and 'E' in a bold, sans-serif font, enclosed within a partial rectangular border.	CE marking of conformity
The consult instructions for use symbol is an icon of an open book.	Consult instructions for use
The manufacturer symbol is an icon of a factory building.	Manufacturer

The product is intended for In Vitro Diagnostic Use. In vivo diagnostic or therapeutic applications are strictly forbidden.

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