Monoclonal Antibody to CD45, PE-Cy™5 conjugated (CD45 PE-Cy5)

Cat.No. ED7067

1. Intended purpose

The reagent CD45 PE-Cy5 permits identification and enumeration of cell populations expressing human CD45 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 CD45 antigen produced by hybridoma clone MEM-28 which was purified by affinity chromatography and labeled with tandem dye R-phycoerythrin-Cy™5 (PE-Cy5). The labeled antibody is diluted at optimum concentration in stabilizing phosphate buffered saline (PBS) solution containing 15mM sodium azide. The content of a vial (1 ml) is sufficient for 100 tests.
The clone MEM-28 was derived from hybridization of mouse myeloma cells with spleen cells from BALB/c mice immunized with human thymocytes and T lymphocytes.

Product specification

Content	100 tests, 1 ml
Usage	10 µl per test
Specificity	Human CD45
Clone	MEM-28
Isotype	Mouse IgG1
Fluorochrome	PE-Cy™5
λ excitation	488 nm
Emission maximum	664 nm

4. Materials required but not provided

Test tubes for blood staining (e.g. 12 × 75 mm)
Commercial lysing solution
Phosphate buffered saline (PBS)

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

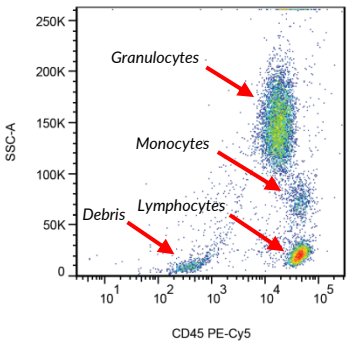
Staining protocol

1. Add 10 µl of CD45 PE-Cy5 reagent to a test tube.
2. Add 100 µl of blood sample to each tube. Vortex the tubes.
3. Incubate tubes for 15 - 20 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 CD45 PE-Cy5 using a flow cytometer. Visualize recorded data using appropriate plot such as side-scatter (SSC) versus PE-Cy™5 intensity as shown in figure 1. All leukocytes are bright (CD45+), nonleukocytes (debris, erythrocytes, platelets, etc.) are dim (CD45-). Set suitable gates for analysis.

Fig. 1: Leukocytes stained with CD45 PE-Cy5 reagent



10. Analytical performance

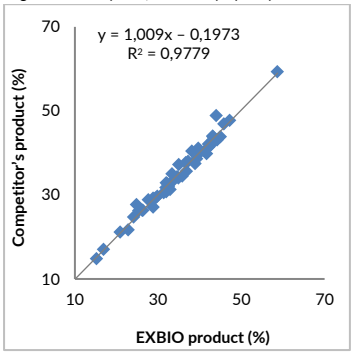
Specificity

The antibody MEM-28 reacts with all alternative forms of human CD45 phosphotyrosine phosphatase (Leukocyte Common Antigen), a 180-220 kDa single chain type I transmembrane protein expressed at high level on all cells of hematopoietic origin, except from erythrocytes and platelets. HLDA III; WS Code NL 833a

Accuracy

The accuracy of the method was studied by the comparison of CD45 PE-Cy5 reagent with competitor's product in parallel staining of 50 blood samples. The regression analysis is given below.

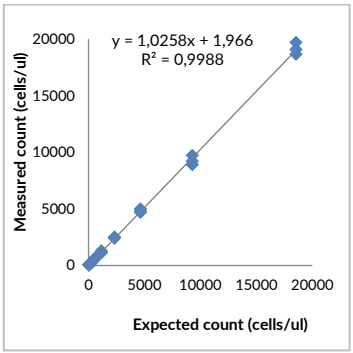
Regression Analysis of CD45+ Lymphocytes



Linearity

The linearity of the method was determined on 10 serial dilutions of leukocyte-enriched blood sample (buffy coat). Cell samples were stained by CD45 PE-Cy5 reagent in triplicates. Measured and expected values were expressed in terms of absolute count (cells/µl) in graphs given below.

Range of CD45+ Lymphocytes



Repeatability

The repeatability of the assay was measured on one blood sample in ten tubes in parallel. Coefficient of variation (CV) is given in the table below.

Leukocyte subset	Unit	n	AVG	SD	CV
Lymphocytes	%	10	48.9	1.07	2.18

Reproducibility

The reproducibility of the assay was measured on stabilized blood sample (Immuno-Troll™ Cells, Beckman-Coulter) under the same conditions for three weeks. Coefficient of variation (CV) is given in the table below.

Leukocyte subset	Unit	n	AVG	SD	CV
Lymphocytes	%	13	22.5	0.73	3.24

11. Clinical performance

Expected values

N/A

12. References

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Stover DR et al. (1991) Protein-tyrosine-phosphatase CD45 is phosphorylated transiently on tyrosine upon activation of Jurkat T cells. Proc Natl Acad Sci USA 88: 7704-7707

Taetle R et al. (1991) Regulation of CD45 expression in human leukemia cells. Leukemia 5: 309-314

Nakano A et al. (1990) Expression of leukocyte common antigen (cd45) on various human leukemia/lymphoma cell lines. Acta Pathol Jpn. 40: 107-15

Yamada A et al. (1990) Effect of activation of protein kinase C on CD45 isoform expression and CD45 protein tyrosine phosphatase activity in T cells. Eur J Immunol. 20: 1655-60

Bazil V et al. (1989) Sialic acid-dependent epitopes of CD45 molecules of restricted cellular expression. Immunogenetics 29: 202-5

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: 23-34

Leukocyte Typing III., McMichael A. J. et al (Eds.), Oxford University Press (1987).

13. Manufacturer

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14. Trademarks

Cy™ and CyDye™ are registered trademarks of Cytiva.
Immuno-Troll™ Cells is registered trademark of Beckman-Coulter.

15. Revision History

• Version 1, ED7067_IFU_v1
Initial Release
• Version 2, ED7067_IFU_v2
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 3, ED7067_IFU_v3
The company logo changed. IFU layout changed. "Keep away from sunlight." – added. Postal code changed:" 25250 Vestec".
• Version 4, ED7067_IFU_v4
In the Trademarks section was changed from GE Healthcare to Cytiva.

Monoclonal Antibody to CD45, PE-Cy™5 conjugated (CD45 PE-Cy5)

IVD CE

Instructions for Use

Version: ED7067_IFU_v4_EN
Date of Issue: 11-01-2021

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