KOMBITEST CD45 FITC / CD14 PE

Cat.No. ED7056

1. Intended purpose

The KOMBITEST CD45 FITC / CD14 PE is designed for establishing an optimal lymphocyte gate for immunophenotyping of erythrocyte-lysed whole blood.

2. Test principle

This test is based on the specific binding of monoclonal antibodies to the antigenic determinants expressed on the surface of leukocytes. The monoclonal antibodies are labeled with different fluorochromes which are excited via laser beam from a flow cytometer during analysis. Subsequent emissions of light from the fluorochromes of each cell are collected and analyzed by a flow cytometer. The fluorescence intensity differences enable the separation of cell subsets based on the expression of analyzed antigens.
The specific staining of blood cells is performed by the 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 a premixed combination of mouse monoclonal antibody against human CD45 antigen (clone MEM-28) labeled with Fluorescein isothiocyanate (FITC), and mouse monoclonal antibody against human CD14 antigen (clone MEM 15) labeled with R-phycoerythrin (PE). Labeled antibodies are 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 50 tests.

Product specification

Content	50 tests, 1 ml	
Usage	20 µl per test	
Specificity	CD45	CD14
Clone	MEM-28	MEM-15
Isotype (mouse)	IgG1	IgG1
Fluorochrome	FITC	PE
λ excitation	488 nm	488 nm
Emission maximum	525 nm	575 nm

4. Materials required but not provided

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

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 CE-IVD 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.
- The flow cytometer should be calibrated on a routine basis using fluorescent microbeads to ensure stable sensitivity of detectors.
- Any non-performance of the 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.
- Concentrations of labeled antibodies in this reagent were optimized to offer the best specific signal/non-specific signal ratio. Therefore, it is important to adhere to the reagent volume/sample volume ratio in every test. Do not dilute the reagent.
- Do not use reagent volumes other than specified in this IFU.
- 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 a hyperleukocytose sample, it is recommended to dilute the 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.

8. Specimen

Use the peripheral human blood in a sterile tube with an anticoagulant (Heparin or EDTA).
Blood must be stored at room temperature.
Use the blood sample no later than 48 hours after collection.

9. Procedure

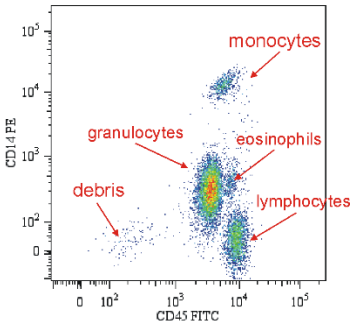
Staining protocol

1. Add 20 µl of KOMBITEST CD45 FITC / CD14 PE reagent to a test tube.
2. Add 100 µl of blood sample to the tube. Vortex the tube.
3. Incubate the tube for 15-20 minutes at room temperature in the dark.
4. Perform lysis of red cells using 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 0.1–0.5 ml of PBS.
9. Analyze the sample immediately using a flow cytometer or store sample at 2-8°C in the dark and analyze within 24 hours provided that cells were fixed.

Flow Cytometric Analysis

Analyze stained samples using a flow cytometer equipped with excitation laser 488 nm and proper filters. Compensate fluorescent signals prior to or after data acquisition.
Following procedure is recommended. However, any other lymphocyte gate assessment procedure may be successful provided that nonlymphocytes (debris, monocytes, granulocytes, etc.) are eliminated using the same principle. Figure 1 shows ungated cell subsets on the CD45 FITC versus CD14 PE dot-plot.

Fig. 1: Leukocytes stained with KOMBITEST CD45 FITC / CD14 PE



1. Visualize ungated data in a Side Scatter (SSC) versus Forward Scatter (FSC) dot-plot and set gate A including all lymphocytes as shown in figure 2.
2. Visualize events gated on the region A as a CD45 FITC versus CD14 PE dot-plot and create a gate B around lymphocytes (figure 3). The gate B discriminates all nonlymphocytes (debris, monocytes, granulocytes, etc.).
3. Visualize events gated on the region B as a Side Scatter (SSC) versus Forward Scatter (FSC) dot-plot as shown in figure 4. Establish an optimal gate for lymphocytes (gate C). Please note that the optimal lymphocyte gate is valid only for data acquired under the same experimental conditions and with the same blood specimen.

Fig. 2: Delimination of all lymphocytes (gate A)

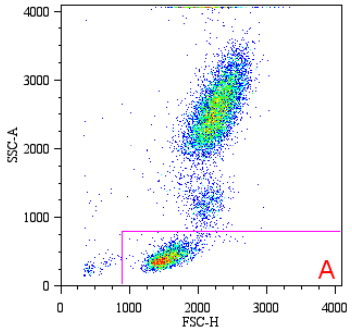


Fig. 3: CD45 vs. CD14, gated on region A

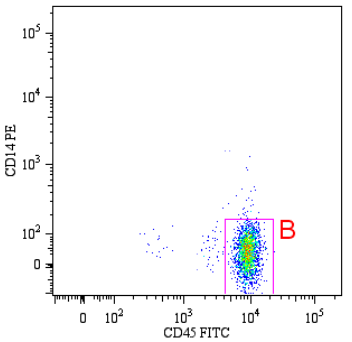
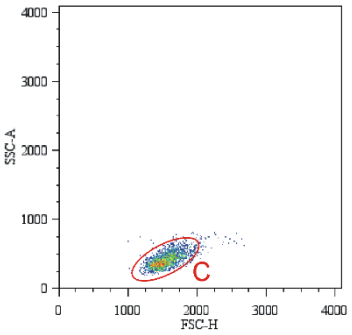


Fig. 4: Optimal gate for lymphocytes (gate C)



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. The monoclonal antibody MEM-28 was assigned to CD45 during the Human Leukocyte Differentiation Antigen workshop (HLDA3 WS Code: NL 833a).
The antibody MEM-15 reacts with CD14, a 53-55 kDa GPI (glycosylphosphatidylinositol) linked membrane glycoprotein expressed on monocytes, macrophages and weakly on granulocytes; it is expressed by most tissue macrophages. This antibody also reacts with soluble forms of CD14 found in serum and in the urine of some nephrotic patients. The monoclonal antibody MEM-15 was assigned to CD14 during the Human Leukocyte Differentiation Antigen workshop (HLDA3 WS Code: M 252).

11. Clinical performance

Expected values

N/A

12. References

Dawes R, Petrova S, Liu Z, Wraith D, Beverley PC, Tchilian EZ. Combinations of CD45 isoforms are crucial for immune function and disease. J Immunol. 2006 Mar 15;176(6):3417-25.

Fernández-Real JM, Broch M, Richart C, Vendrell J, López-Bermejo A, Ricart W: CD14 monocyte receptor, involved in the inflammatory cascade, and insulin sensitivity. J Clin Endocrinol Metab. 2003 Apr;88(4):1780-4.

Horejsi V, Angelisova P, Bazil V, Kristofova H, Stoyanov S, Stefanova I, Hausner P, Vosecky M, Hilgert I: 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). 1988;34(1):23-34.

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

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

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

Leukocyte Typing VI., Kishimoto T. et al. (Eds.), Garland Publishing Inc. (1997).

13. Manufacturer

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14. Trademarks

N/A

15. Revision History

• Version 1, ED7056_IFU_v1
Initial Release
• Version 2, ED7056_IFU_v2
The text removed in in the reagent provided: "0.2% (w/v) high-grade protease free Bovine Serum Albumin (BSA) as a stabilizing agent."
• Version 3, ED7056_IFU_v3
The company logo changed. IFU layout changed. "Keep away from sunlight", "Do not aliquot" added in Storage section. Texts "Do not dilute the reagent" and "Do not use reagent volumes other than specified in this IFU" were added in warning section. Postal code changed:" 25250 Vestec".

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50 tests | Cat.No. **ED7056**

IVD CE

Instructions for Use

Version: ED7056_IFU_v3_EN
Date of Issue: 13-12-2020

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