

KOMBITEST

CD3 FITC/

CD16 + CD56 PE

Cat.No. ED7054

1. Intended purpose

The KOMBITEST CD3 FITC / CD16 + CD56 PE is designed for identification and enumeration of mature human natural killer (NK) and T lymphocytes in erythrocyte-lysed whole blood using Flow Cytometry.

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 CD3 antigen labeled with Fluorescein isothiocyanate (FITC), mouse monoclonal antibody against human CD16 antigen labeled with R-phycoerythrin (PE), and mouse monoclonal antibody against human CD56 antigen 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	CD3	CD16	CD56
Clone	UCHT1	3G8	MEM-188
Isotype (mouse)	IgG1	IgG1	IgG2a
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)
EXCELLYSE Easy lysing solution
(Cat.No. ED7066)

5. Equipment required

Automatic pipettes with disposable tips
Vortex mixer
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 labels 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 reagents after their 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 (NaN₃) 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 × 10⁶ 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

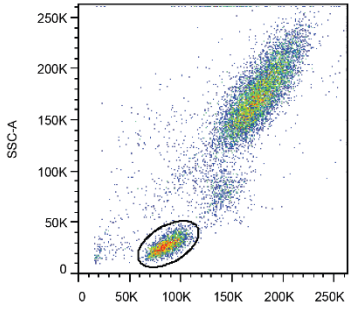
Staining protocol

- Add 20 µl of KOMBITEST CD3 FITC / CD16 + CD56 PE reagent to a test tube.
- Add 100 µl of blood sample to the tube. Vortex the tube.
- Incubate the tube for 15-20 minutes at room temperature in the dark.
- Perform lysis of red cells using EXCELLYSE Easy lysing solution (Cat.No. ED7066) or any other commercial lysing solution containing formaldehyde as a fixative using Lyse/Wash protocol. Follow the instructions of the lysing solution manufacturer.
- 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

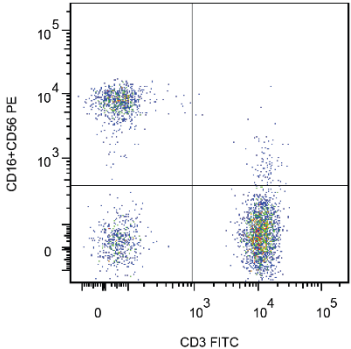
Analyze stained samples using a flow cytometer with excitation laser 488 nm and proper filters. Compensate fluorescent signals prior to or after data acquisition. Visualize compensated data on the side-scatter (SSC) versus forward-scatter (FSC) plot. Set the gate for lymphocyte population (Figure 1). Alternatively, set the optimal lymphocyte gate using KOMBITEST CD45 FITC / CD14 PE (refer the datasheet for lymphocyte gate assessment procedure).

Fig. 1: Delimitation of lymphocyte population



Then make a CD3 FITC versus CD16 + 56 PE dot-plot of lymphocyte population (Figure 2). Separate populations using appropriate gate and calculate the percentage of natural killer (NK) lymphocytes situated in upper-left quadrant (CD3-CD16+56+) and T lymphocytes situated in lower-right quadrant (CD3+CD16-56-) on the dot-plot.

Fig. 2: Lymphocytes in a dot-plot CD16+CD56 PE vs. CD3 FITC



10. Analytical performance

Specificity

The antibody UCHT1 recognizes the CD3 antigen of the TCR/CD3 complex on mature human T cells. The UCHT1 antibody reacts with the epsilon chain of the CD3 complex.
HLDA I; WS Code T 3
HLDA II; WS Code T 126
HLDA III; WS Code T 471
HLDA VI; WS Code T 6T-CD3.1

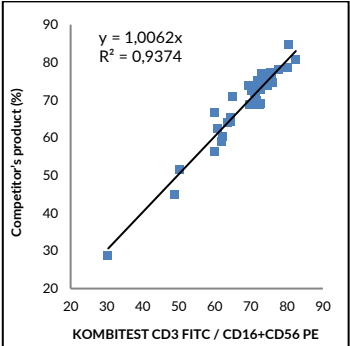
The antibody 3G8 recognizes CD16, a low affinity receptor for aggregated IgG (FcγRIII antigen). CD16 exists in two different isoforms: CD16a (FcγRIIIA; 50-65 kDa; expressed on NK-cells, monocytes and macrophages) and CD16b (FcγRIIIB; 48 kDa; mainly expressed on neutrophils).
HLDA V; WS Code NK80

The antibody MEM-188 reacts with a 180 kDa isoform of CD56 (NCAM) characteristic for leukocytes. CD56 is a single transmembrane glycoprotein of immunoglobulin supergene family; the molecular weights of different isoforms ranging from 135 to 220 kDa.
HLDA VI; WS Code AS A055, NK NK26

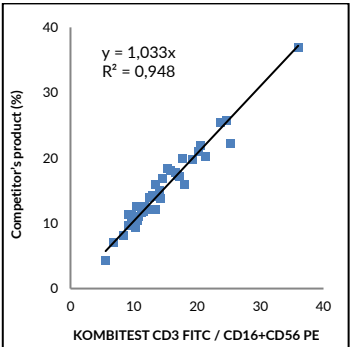
Accuracy

The accuracy of the method was studied by the comparison of KOMBITEST CD3 FITC / CD16 + CD56 PE with competitor's product in parallel staining of 43 blood samples. The regression analyzes are given below.

Regression Analysis of CD3*CD16 CD56 Lymphocytes



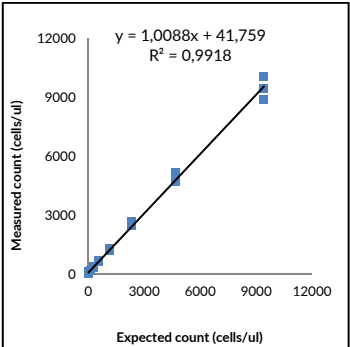
Regression Analysis of CD3*CD16*CD56 Lymphocytes



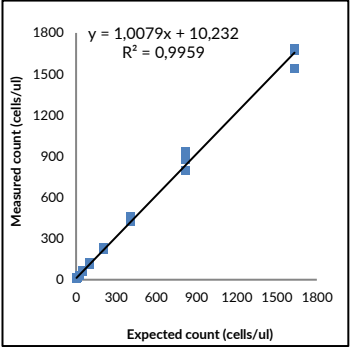
Linearity

The linearity of the method was verified on 10 serial dilutions of leukocyte-enriched blood sample (buffy coat). Cell samples were stained by KOMBITEST CD3 FITC / CD16 + CD56 PE in triplicates. Measured and expected values were expressed in terms of absolute count (cells/µl) in graphs given below.

Range of CD3*CD16 CD56 Lymphocytes



Range of CD3-CD16+CD56+ Lymphocytes



Repeatability

The repeatability of the assay was measured on stabilized blood sample (Immuno-Troll™ Cells, Beckman-Coulter) in ten tubes in parallel. Coefficients of variation (CV) are given in the table below.

Lymphocyte Subset	Unit	n	Average	SD	CV
CD3+CD16-CD56-	%	10	70.3	0.94	1.34
CD3-CD16+CD56+	%	10	11.9	0.84	7.02

Reproducibility

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

Lymphocyte Subset	Unit	n	Average	SD	CV
CD3+CD16-CD56-	%	14	72.7	0.97	1.34
CD3-CD16+CD56+	%	14	11.7	0.87	7.43

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.

Lymphocyte Subset	Unit	n	Mean	95% Range
CD3+CD16-CD56-	%	108	71	52-83
	cells/µl	54	1480	886-2341
CD3-CD16+CD56+	%	108	15	6-34
	cells/µl	54	319	121-536

12. References

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13. Manufacturer

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14. Trademarks

Immuno-Troll™ Cells is registered trademark of Beckman-Coulter.

15. Revision History

- Version 1, ED7054_IFU_v1

Initial Release

- Version 2, ED7054_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, ED7054_IFU_v3

The company logo changed, IFU layout changed. "Keep away from sunlight", "Do not aliquote" and "Expiration date is stated on a vial labels and on outer packaging" added in Storage section.

"Do not dilute the reagent" and "Do not use reagent volumes other than specified in this IFU" added in warning section.

Trademarks section added.

Staining protocol section changed - using of Excellyse Easy lysing solution (Cat.No. ED7066) added, washing steps after red cell lysis (steps 5 - 8 in the previous version) removed.

- Version 4, ED7054_IFU_v4

References were added. Product Use Limitation text was refined.

exbio

KOMBITEST CD3 FITC / CD16 + CD56 PE

50 tests | Cat.No. ED7054



Instructions for Use

Version: ED7054_IFU_v4_EN

Date of Issue: 04-02-2020

EN

Symbols



Catalogue number



Batch code



Use-by date



Temperature limits



Keep away from sunlight



In vitro diagnostic medical device



CE marking of conformity



Consult instructions for use



Manufacturer

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

Products shall not be used for resale or transfer to third parties either as a stand-alone product or as a manufacture component of another product without written consent of EXBIO Praha, a.s. EXBIO Praha, a.s. will not be held responsible for patent infringement or any other violations of intellectual property rights that may occur with the use of the products. Orders for all products are accepted subject to the Term and Conditions available at www.exbio.cz. EXBIO, EXBIO Logo, and all other trademarks are property of EXBIO Praha, a.s..