KOMBITEST CD3 FITC/ CD8 PE / CD45 PerCP/ CD4 APC

Cat.No. ED7045

1. Intended purpose

Intended use

The KOMBITEST CD3 FITC / CD8 PE / CD45 PerCP / CD4 APC is designed for identification and enumeration of mature human helper/inducer (CD3+CD4+) and suppressor/cytotoxic (CD3+CD8+) T lymphocytes in erythrocyte-lysed whole blood using Flow Cytometry. The helper/suppressor ratio (CD4+ / CD8+) may also be determined.

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 (clone UCHT1) labeled with Fluorescein isothiocyanate (FITC), mouse monoclonal antibody against human CD8 antigen (clone MEM-31) labeled with R-phycoerythrin (PE), mouse monoclonal antibody against human CD45 antigen (clone MEM-28) labeled with Peridinin-chlorophyll-protein complex (PerCP), and mouse monoclonal antibody against human CD4 antigen (clone MEM-241) labeled with Allophycocyanin (APC). 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	CD8	CD45	CD4
Clone	UCHT1	MEM-31	MEM-28	MEM-241
Isotype (mouse)	IgG1	IgG2a	IgG1	IgG1
Fluorochrome	FITC	PE	PerCP	APC
λ excitation	488 nm	488 nm	488 nm	633 nm
Emission maximum	525 nm	575 nm	670 nm	660 nm

4. Materials required but not provided

Suitable 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 two lasers (488 nm and 633 or 635 nm)

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 (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

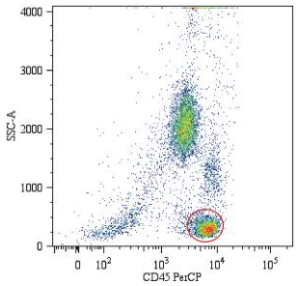
Staining protocol

1. Add 20 µl of KOMBITEST CD3 FITC / CD8 PE / CD45 PerCP / CD4 APC reagent to a test tube.
2. Add 50 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 EXCELLYSE Easy lysing solution (Cat.No. ED7066) or any other commercial lysing solution containing formaldehyde as a fixative. Follow the instructions of the lysing solution manufacturer.
5. 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 two excitation lasers (488 nm and 633 or 635 nm) and proper filters. Compensate fluorescent signals prior to or after data acquisition.
Visualize compensated data on the side-scatter (SSC) versus CD45 PerCP plot. Set the gate for CD45+ lymphocyte population as shown in Figure 1.

Fig. 1 Delimitation of CD45+ lymphocyte population



Visualize CD45+ lymphocytes in a dot-plot CD8 PE versus CD3 FITC as shown in Figure 2. Separate populations using appropriate gates and calculate the percentage of suppressor/cytotoxic T lymphocytes situated in upper-right quadrant (CD3+CD8+ subpopulation) on the dot-plot.

Visualize CD45+ lymphocytes in a dot-plot CD4 APC versus CD3 FITC as shown in Figure 3. Separate populations using appropriate gates and calculate the percentage of helper/inducer T lymphocytes situated in upper-right quadrant (CD3+CD4+ subpopulation) on the dot-plot.

Fig. 2 CD45+ lymphocytes in a dot-plot CD8 PE vs. CD3 FITC

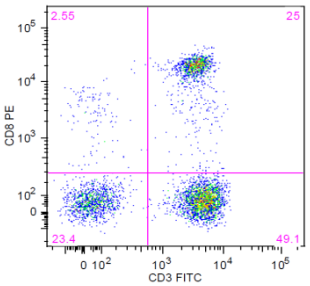
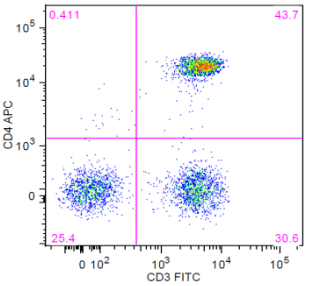


Fig. 3 CD45+ lymphocytes in a dot-plot CD4 APC vs. CD3 FITC



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 III; WS Code T 126
HLDA III; WS Code T 471
HLDA VI; WS Code T 6T-CD3.1

The antibody MEM-31 recognizes a conformationally-dependent epitope of CD8, a cell surface glycoprotein that mediates efficient cell-cell interactions within the immune system. CD8 is a disulfide-linked dimer (each monomer approx. 32-34 kDa) and exists as a CD8α/α homodimer on subsets of memory T cells, intraepithelial lymphocytes, NK cells and dendritic cells, or as a CD8α/β heterodimer on majority of MHC I-restricted cytotoxic T cells and thymocytes.
HLDA III; WS Code T 575

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

The antibody MEM-241 recognizes CD4 co-receptor, a 55 kDa transmembrane glycoprotein of immunoglobulin family expressed on subsets of T lymphocytes (such as "helper" T cells, CD4+ regulatory T cells or CD4+CD8+ double-positive T cells) and also on monocytes, tissue macrophages and granulocytes. HLDA VIII (HCDM); WS Code M241

Accuracy

The accuracy of the method was studied by the comparison of KOMBITEST CD3 FITC / CD8 PE / CD45 PerCP / CD4 APC with competitor's product in parallel staining of blood samples. Samples were analyzed using BD FACSCanto™ Flow Cytometer. The regression parameters are given in the table below.

Lymphocyte Subset	Unit	n	Slope	Intercept	r2	Range
CD3+	%	108	0.986	1.55	0.954	38-91
	cells/µl	54	1.003	-7.05	0.942	573-2571
CD3+CD8+	%	108	0.978	0.66	0.954	6-48
	cells/µl	54	1.077	-32.7	0.951	133-1242
CD3+CD4+	%	108	0.979	0.89	0.964	17-68
	cells/µl	54	0.992	-1.17	0.953	331-1682

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 / CD8 PE / CD45 PerCP / CD4 APC in triplicates. Measured data were observed to be linear across the tested range 33-17022 lymphocytes/µl. Cell subsets were in following ranges.

Lymphocyte Subset	Range (cells/µl)
CD3+	25-13034
CD3+CD8+	7-3674
CD3+CD4+	15-7512

Repeatability

The repeatability of the assay was measured on one blood sample in twelve tubes in parallel. Coefficients of variation (CV) are given in the table below.

Lymphocyte Subset	Unit	n	Average	SD	CV
CD3+	%	12	46.2	1.17	2.52
	cells/µl	12	589	32.1	5.46
CD3+CD8+	%	12	18.6	0.66	3.56
	cells/µl	12	237	14.1	5.93
CD3+CD4+	%	12	25.9	0.90	3.46
	cells/µl	12	330	18.7	5.67

Reproducibility

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

Lymphocyte Subset	Unit	n	Average	SD	CV
CD3+	%	42	73.9	1.12	1.51
	cells/µl	42	966	39.3	4.07
CD3+CD8+	%	42	26.8	0.95	3.55
	cells/µl	42	350	16.0	4.55
CD3+CD4+	%	42	44.9	0.96	2.15
	cells/µl	42	587	27.6	4.70

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+	%	108	70	52-83
	cells/µl	54	1475	796-2363
CD3+CD8+	%	108	25	13-46
	cells/µl	54	503	208-1031
CD3+CD4+	%	108	44	25-64
	cells/µl	54	939	473-1524

12. References

This product has not been published yet.

13. Manufacturer

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14. Trademarks

BD FACSCanto™ is registered trademark of Becton, Dickinson and Company, Immuno-Trol™ Cells is registered trademark of Beckman-Coulter

15. Revision History

• Version 1, ED7045_IFU_v1
Initial Release
• Version 2, ED7045_IFU_v2
A new text added in the Precautions section : "Intended for In Vitro Diagnostic use in laboratories outside USA and Canada. This CE-IVD kit is in conformity with the European In Vitro Diagnostic Medical Device Directive 98/79/EC".
• Version 3, ED7045_IFU_v3
Using of Excellyse Easy lysing solution (Cat.No. ED7066) added
Results table for accuracy and linearity added (previous graphs removed)
Trademarks section added
References removed
• Version 4, ED7045_IFU_v4
Change of dot-plot for Figure 2 and Figure 3
• Version 5, ED7045_IFU_v5
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 6, ED7045_IFU_v6
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.
• Version 7, ED7045_IFU_v7
Product Use Limitation text was refined.

KOMBITEST CD3 FITC / CD8 PE / CD45 PerCP / CD4 APC

50 tests | Cat.No. **ED7045**

IVD CE

Instructions for Use

Version ED7045_IFU_v7_EN
Date of Issue: 04-02-2020

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