KOMBITEST
CD3 FITC/ CD8 PE
Cat.No. ED7051

1. Intended purpose

The KOMBITEST CD3 FITC / CD8 PE is designed for identification and enumeration of mature human suppressor / cytotoxic (CD3+CD8+) 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 (clone UCHT1) labeled with Fluorescein isothiocyanate (FITC) and mouse monoclonal antibody against human CD8 antigen (clone MEM-31) 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	CD8
Clone	UCHT1	MEM-31
Isotype (mouse)	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 (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

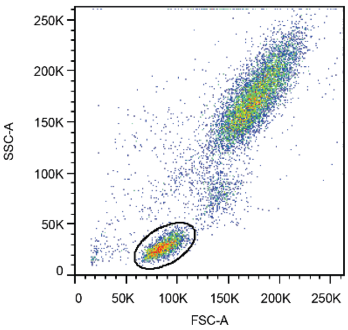
Staining protocol

1. Add 20 µl of KOMBITEST CD3 FITC / CD8 PE 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 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.
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

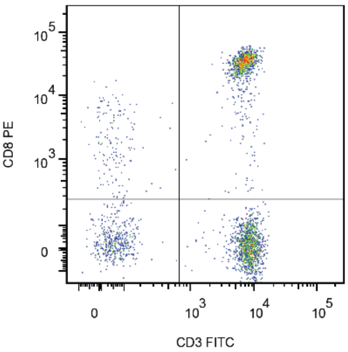
Analyze stained samples using a flow cytometer with excitation laser 488 nm and proper filters. Compensate fluorescent signals prior to or after data acquisition.
Visualize compensated data on the side-scatter (SSC) versus forward-scatter (FSC) plot. Set the gate for lymphocyte population (Figure 1). 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 CD8 PE dot-plot of lymphocyte population (Figure 2). Separate populations using appropriate gate and calculate the percentage of suppressor / cytotoxic T lymphocytes situated in upper-right quadrant (CD3+CD8+ subpopulation) on the dot-plot.

Fig. 2: Lymphocytes in a dot-plot CD8 PE vs. CD3 FITC



10. Analytical performance

Specificity

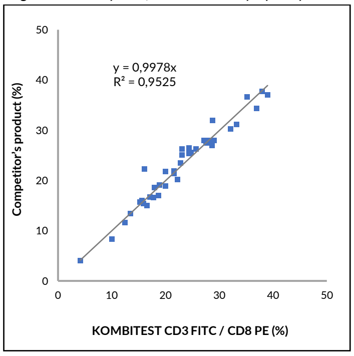
The antibody UCHT1 recognizes the CD3 antigen of the TCR/CD3 complex on mature human T cells. The UCHT1 antibody reacts with the epsilon chain of the CD3 complex.
HLDA I; WS Code T 3
HLDA III; WS Code T 126
HLDA III; WS Code T 471
HLDA VI; WS Code T 6T-CD3.1

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

Accuracy

The accuracy of the method was studied by the comparison of KOMBITEST CD3 FITC / CD8 PE with competitor's product in parallel staining of 43 blood samples. The regression analysis is given below.

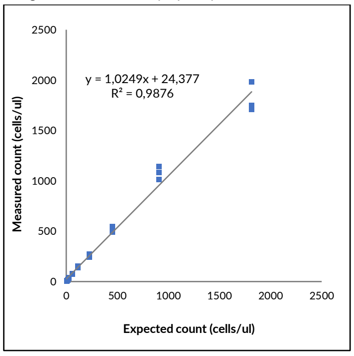
Regression Analysis of CD3+CD8+ Lymphocytes



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 / CD8 PE in triplicates. Measured and expected values were expressed in terms of absolute count (cells/µl) in graphs given below.

Range of CD3+CD8+ Lymphocytes



Repeatability

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

Lymphocyte Subset	Unit	n	Average	SD	CV
CD3+CD8+	%	10	25.5	1.81	7.10

Reproducibility

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

Lymphocyte Subset	Unit	n	Average	SD	CV
CD3+CD8+	%	14	26.0	1.60	6.15

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+CD8+	%	108	25	13-46

12. References

Alarcón B, Swamy M, van Santen HM, Schamel WW: T-cell antigen-receptor stoichiometry: pre-clustering for sensitivity. EMBO Rep. 2006 May;7(5):490-5.

Arnett KL, Harrison SC, Wiley DC: Crystal structure of a human CD3-epsilon/delta dimer in complex with a UCHT1 single-chain antibody fragment. Proc Natl Acad Sci U S A. 2004 Nov 16;101(46):16268-73.

Barclay, Brown et al.: The Leukocyte Antigen FactsBook, 2nd edition, Harcourt Brace & Company, London, (1997).

Brdičková N, Brdicka T, Angelisová P, Horváth O, Spicka J, Hilgert I, Paces J, Simeoni L, Kliche S, Merten C, Schraven B, Horejší V: LIME: a new membrane Raft-associated adaptor protein involved in CD4 and CD8 coreceptor signaling. J Exp Med. 2003 Nov 17;198(10):1453-62.

Devine L, Thakral D, Nag S, Dobbins J, Hodsdon ME, Kavathas PB: Mapping the binding site on CD8 beta for MHC class I reveals mutants with enhanced binding. J Immunol. 2006 Sep 15;177(6):3930-8.

Drbal K, Moertelmaier M, Holzhauser C, Muhammad A, Fuertbauer E, Howorka S, Hinterberger M, Stockinger H, Schütz GJ: Single-molecule microscopy reveals heterogeneous dynamics of lipid raft components upon TCR engagement. Int Immunol. 2007 May;19(5):675-84.

Huang Y, Wange RL: T cell receptor signaling: beyond complex complexes. J Biol Chem. 2004 Jul 9;279(28):28827-30.

Kuhns MS, Davis MM, Garcia KC: Deconstructing the form and function of the TCR/CD3 complex. Immunity. 2006 Feb;24(2):133-9.

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

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

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

Pang DJ, Hayday AC, Bijlmakers MJ.: CD8 Raft localization is induced by its assembly into CD8alpha beta heterodimers, Not CD8alpha alpha homodimers. J Biol Chem. 2007 May 4;282(18):13884-94.

Rieux-Laucat F, Hivroz C, Lim A, Mateo V, Pellier I, Selz F, Fischer A, Le Deist F: Inherited and somatic CD3zeta mutations in a patient with T-cell deficiency. N Engl J Med. 2006 May 4;354(18):1913-21.

Torres PS, Alcover A, Zapata DA, Arnaud J, Pacheco A, Martín-Fernández JM, Villasevil EM, Sanal O, Regueiro JR: TCR dynamics in human mature T lymphocytes lacking CD3 gamma. J Immunol. 2003 Jun 15;170(12):5947-55.

van den Berg HA, Wooldridge L, Laugel B, Sewell AK: Coreceptor CD8-driven modulation of T cell antigen receptor specificity. J Theor Biol. 2007 Nov 21;249(2):395-408.

13. Manufacturer

EXBIO Praha, a.s.
Nad Safinou II 341
25250 Vestec
Czech Republic

info@exbio.cz
technical@exbio.cz
orders@exbio.cz
www.exbio.cz

14. Trademarks

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

15. Revision History

- Version 1, ED7051_IFU_v1
Initial Release
- Version 2, ED7051_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, ED7051_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, ED7051_IFU_v4
References were added. Product Use Limitation text was refined.

KOMBITEST CD3 FITC / CD8 PE

50 tests | Cat.No. **ED7051**

IVD CE

Instructions for Use

Version ED7051_IFU_v4_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