

KOMBITEST CD4 FITC / CD8 PE

Cat.No. ED7052

1. Intended purpose

The KOMBITEST CD4 FITC / CD8 PE is designed for percentage enumeration of mature human helper/inducer (CD4+) and suppressor/cytotoxic (CD8+) 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 CD4 antigen (clone MEM-241) 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	CD4	CD8
Clone	MEM-241	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)
PBS buffer
Deionized water

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 (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

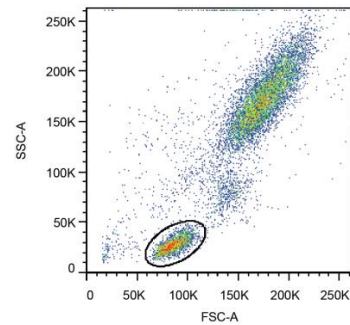
- Add 20 µl of KOMBITEST CD4 FITC / CD8 PE reagent to a test tube.
- Add 100 µl of blood sample to the tube. Vortex the tube.
- Incubate the tube for 15 - 20 minutes at room temperature in the dark.
- Perform lysis of red cells using EXCELLYSE Easy lysing solution (Cat.No. ED7066) or any other commercial lysing solution containing formaldehyde as a fixative. Follow the instructions of the lysing solution manufacturer.
- Centrifuge tubes for 5 minutes at 300 g.
- Remove supernatant and resuspend pellet with 3-4 ml of PBS.
- Centrifuge tubes for 5 minutes at 300 g.
- Remove supernatant and resuspend pellet with 0.1 - 0.5 ml of PBS.
- 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.

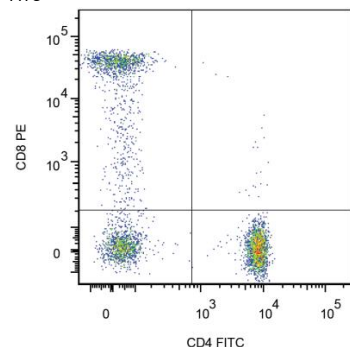
Visualize compensated data on the side-scatter (SSC) versus forward-scatter (FSC) plot. Set the gate for lymphocyte population (Figure 1). Alternatively, set the optimal lymphocyte gate using KOMBITEST CD45 FITC / CD14 PE (Cat.No. ED7056, refer to the datasheet for lymphocyte gate assessment procedure).

Fig. 1: Delimitation of lymphocyte population



Then make a CD4 FITC versus CD8 PE dot-plot of lymphocyte population (Figure 2). Separate populations using appropriate gate and calculate the percentage of helper/inducer lymphocytes situated in lower-right quadrant (CD4+CD8-) and suppressor/cytotoxic lymphocytes situated in upper-left quadrant (CD4-CD8+) on the dot-plot.

Fig. 2: Lymphocytes in a dot-plot CD8 PE vs. CD4 FITC



10. Analytical performance

Specificity

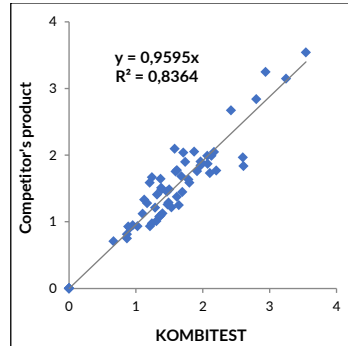
The antibody MEM-241 recognizes CD4 coreceptor, a 55 kDa transmembrane glycoprotein of immunoglobulin family expressed on subsets of T lymphocytes (such as "helper" T cells, CD4+ regulatory T cells or CD4+CD8+ double-positive T cells) and also on monocytes, tissue macrophages and granulocytes. The monoclonal antibody MEM-241 was assigned to CD4 during the Human Leukocyte Differentiation Antigen workshop (HLDA8 (HCDM) WS Code: M241).

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. The monoclonal antibody MEM-31 was assigned to CD8 during the Human Leukocyte Differentiation Antigen workshop (HLDA3 WS Code: T 575).

Accuracy

The accuracy of the method was studied by the comparison of KOMBITEST CD4 FITC / CD8 PE with competitor's product in parallel staining of 61 blood samples. The regression analysis is given below.

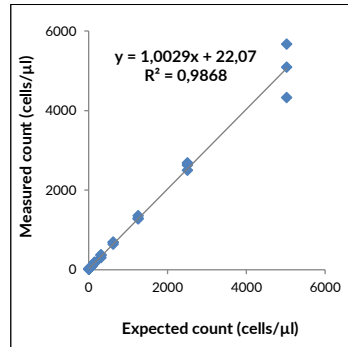
Regression Analysis of CD4/CD8 ratios



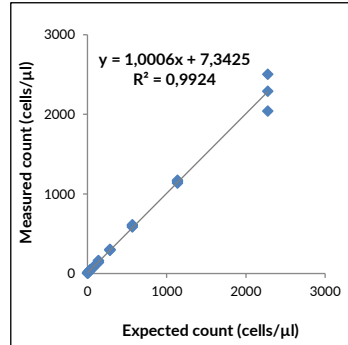
Linearity

The linearity of the method was determined on 10 serial dilutions of leukocyte-enriched blood sample (buffy coat). Cell samples were stained by KOMBITEST CD4 FITC / CD8 PE in triplicates. Measured and expected values were expressed in terms of absolute count (cells/µl) in graphs given below.

Range of CD4+CD8- Lymphocytes



Range of CD4+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	n	AVG	SD	CV
CD4+CD8- (%)	10	42.7	1.27	2.97
CD4+CD8+ (%)	10	30.7	1.12	3.65

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	n	AVG	SD	CV
CD4+CD8- (%)	14	42.9	1.87	4.36
CD4+CD8+ (%)	14	30.5	1.04	3.43

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	n	Mean	95% Range
CD4+CD8- (%)	61	44	27-61
CD4+CD8+ (%)	61	28	14-43
CD4/CD8 ratio	61	1.7	0.8-3.5

12. References

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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, ED7052_IFU_v1
Initial Release

- Version 2, ED7052_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, ED7052_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.

Staining protocol specified to the use of
Excellyse Easy lysing solution (Cat.No. ED7066).

exbio

KOMBITEST CD4 FITC / CD8 PE

50 tests | Cat.No. ED7052



Instructions for Use

Version: ED7052_IFU_v3_EN

Date of Issue: 18-06-2020

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..