KOMBITEST FITC/PE Negative Control

Cat.No. ED7032

1. Intended purpose

KOMBITEST FITC/PE Negative Control is designed for setting a boundary between negatively and positively stained lymphocytes when peripherial blood samples are analyzed using two-color (FITC/PE) KOMBITEST reagents.

2. Test principle

Besides the specific binding of murine antibodies to the antigens, antibodies may also bind to human leukocytes through non-antigen (non-specific) means. This nonspecific binding of monoclonal antibodies may significantly aggravate the resolution between positive and negative fluorescent populations. By showing murine antibodies binding to human leukocytes through non-antigen (non-specific) means, the control sample enables establishing a boundary between negatively and positively stained leukocytes.

3. Reagents provided

The reagent contains premixed combination of mouse monoclonal antibody PPV-04 labeled with Fluorescein isothiocyanate (FITC), and mouse monoclonal antibody PPV-04 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	Undefined epitope on a plant pathogen	
Clone	PPV-04	PPV-04
Isotype (mouse)	IgG2a	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)
Commercial lysing solution
Phosphate buffered saline (PBS)

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 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.
- Any non-performance of 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.
- 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 hyperleukocytose sample, it is recommended to dilute 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.
- Red blood cells from abnormal patients may be resistant to lysis using lysing solutions.
- Blood samples should be stained and analyzed within 24 hours from the blood collection.

8. Specimen

Use the peripheral human blood in a sterile tube with an anticoagulant (Heparin or EDTA).
Blood must be stored at room temperature.

9. Procedure

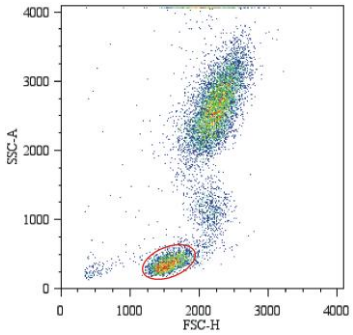
Staining protocol

1. Add 20 µl of KOMBITEST FITC/PE Negative Control to a test tube
2. Add 100 µl of blood sample to each tube. Vortex the tubes.
3. Incubate tubes for 20 - 30 minutes at room temperature in the dark.
4. Perform lysis of red cells using lysing solution. It is recommended to use a 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 0.3 – 0.5 ml of PBS.
9. Analyze samples immediately using flow cytometer or store samples at 2 - 8 °C in the dark and analyze within 24 hours provided that cells were fixed.

Flow Cytometric Analysis

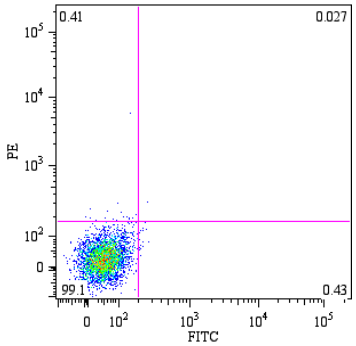
Analyze the control sample stained with KOMBITEST™ FITC/PE Negative Control using a flow cytometer. Compensate fluorescent signals prior to or after data acquisition. Visualize compensated data in the side-scatter (SSC) versus forward-scatter (FSC) plot. Set the gate for lymphocyte population as shown in figure 1.

Fig. 1: Delimitation of lymphocyte population



Visualize lymphocytes in a dot-plot PE (FL2) versus FITC (FL1) as shown in figure 2. Set the gate corresponding to the negative population.

Fig. 2: Negative population of lymphocytes in a dot-plot PE vs. FITC



10. Analytical performance

Specificity

The monoclonal antibody PPV-04 reacts with undefined epitope on a plant pathogen. The antigen is not present on human leukocytes. When added to human whole blood, the antibody PPV-04 does not bind specifically to antigens on the surface of human leukocytes.

11. Clinical performance

N/A

12. References

N/A

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

N/A

15. Revision History

• Version 1, ED7032_IFU_v1
Initial Release
• Version 2, ED7032_IFU_v2
The address was changed: "Nad Safinou II 341".
• Version 3, ED7032_IFU_v3
Precautions section was changed – "Intended for professional use only." – removed. "Intended for In Vitro Diagnostic use in laboratories outside USA and Canada. This CE-IVD reagent is in conformity with the European In Vitro Diagnostic Medical Device Directive 98/79/EC." – added.
• Version 4, ED7032_IFU_v4
Reagent provided section was changed: text "stabilizing" added, "solution" – added and "0.2% (w/v) high-grade protease free Bovine Serum Albumin (BSA) as a stabilizing agent" – removed.
• Version 5, ED7032_IFU_v5
The company logo changed. IFU layout changed. "Keep away from sunlight." – added. Postal code changed:" 25250 Vestec".

KOMBITEST FITC/PE Negative Control

50 tests | Cat.No. **ED7032**

IVD CE

Instructions for Use

Version: ED7032_IFU_v5_EN
Date of Issue: 31-08-2020

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