Monoclonal Antibody to CD63, FITC conjugated (CD63 FITC)

Cat.No. ED7031

1. Intended purpose

The reagent CD63 FITC permits identification and enumeration of cell populations expressing human CD63 antigen in whole blood using flow cytometry. This reagent is widely used for investigation of allergen induced activation of basophils.

2. Test principle

This test is based on specific binding of monoclonal antibody to the antigenic determinant expressed on the surface of leukocytes. The monoclonal antibody is labeled with fluorochrome which is excited via laser beam from a flow cytometer during analysis. Subsequent emission of light from fluorochromes of each cell is collected and analyzed by a flow cytometer. The fluorescence intensity differences enable separation of cell subsets based on expression of analyzed antigen.

Specific staining of blood cells is performed by 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 mouse monoclonal antibody against human CD63 antigen (clone MEM-259) which was purified by affinity chromatography and labeled with Fluorescein isothiocyanate (FITC). The labeled antibody is diluted in an optimal concentration in stabilizing phosphate buffered saline (PBS) solution containing 15mM sodium azide. The content of a vial (2 ml) is sufficient for 100 tests.

Product specification

Specificity	Human CD63
Fluorochrome	FITC
Clone	MEM-259
Isotype	Mouse IgG1
Content	100 tests, 2 ml
Usage	20 µl per test
λ excitation	488 nm
Emission maximum	525 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 aliquote. 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 kit 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.
Use the blood sample no later than 48 hours after collection.

9. Procedure

Staining protocol

1. Add 20 µl CD63 FITC reagent to a test tube.
2. Add 100 µl of blood sample to the tube. Vortex the tube.
3. Incubate the tube 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 the tube for 5 minutes at 300 g.
6. Remove supernatant and resuspend pellet with 3-4 ml of PBS.
7. Centrifuge the tube for 5 minutes at 300 g.
8. Remove supernatant and resuspend pellet with 0.3 – 0.5 ml of PBS.
9. 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 the sample stained with CD63 FITC using a flow cytometer. Make appropriate plots and gates to analyze populations expressing human CD63 antigen.

Example data show an analysis of blood sample from patient with the allergy to bee venom. The test sample (figure 1) was stained using CD63 FITC and CD203c PE after the stimulation with bee venom extract and interleukin-3. The control sample (figure 2) was not stimulated with the allergen.

Fig. 1: Sample from patient with allergy to bee venom, stained using CD63 FITC and CD203c PE after the stimulation with bee venom extract and IL-3.

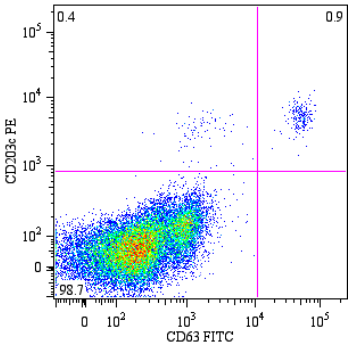
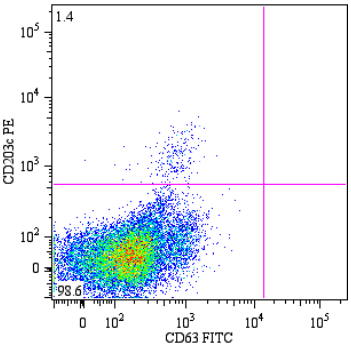


Fig. 2: : Control sample from patient with allergy to bee venom, stained using CD63 FITC and CD203c PE without the stimulation with the allergen.



10. Analytical performance

Specificity

The antibody MEM-259 reacts with CD63 (LAMP-3, lysosome-associated membrane protein-3), a 40-60 kDa glycoprotein of tetraspanin family, and is present in late endosomes, lysosomes and secretory vesicles of various cell types. It is also present in the plasma membrane, usually following cell activation. Hence, it has become a widely used basophil activation marker.

Accuracy

N/A

Linearity

N/A

Repeatability

N/A

Reproducibility

N/A

11. Clinical performance

Expected values

N/A

12. References

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13. Manufacturer

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14. Trademarks

N/A

15. Revision History

• Version 1, ED7031_IFU_v1
Initial Release
• Version 2, ED7031_IFU_v2
The address was changed: "Nad Safinou II 341"
• Version 3, ED7031_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, ED7031_IFU_v4
Precautions section was changed – stabilizing added, solution – added and "0.2% (w/v) high-grade protease free Bovine Serum Albumin (BSA) as a stabilizing agent" – removed.
• Version 5, ED7031_IFU_v5
The company logo changed. IFU layout changed.
"Keep away from sunlight." – added.
"Blood must be stored at room temperature. Use the blood sample no later than 48 hours after collection." - added.
The address was changed:" 25250 Vestec"

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100 tests | Cat.No. **ED7031**

IVD CE

Instructions for Use

Version: ED7031_IFU_v5_EN
Date of Issue: 18-02-2020

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