

IOtest®
Glycophorin A-PE

REF A07792
100 tests; 2 mL
20 µL / test



IOtest
Conjugated Antibody



ENGLISH	Specifications
Specificity	Glycophorin A (CD235a)
Clone	11E4B-7-6 (KC16)
Hybridoma	NS/1-Ag4 x Balb/c
Immunogen	Human red blood cells
Immunoglobulin	IgG1
Species	Mouse
Source	Ascites
Purification	Protein A affinity chromatography
Fluorochrome	R Phycoerythrin (PE)
λ excitation	488 nm
Peak emission	575 nm
Buffer	PBS pH 7.2 plus 2 mg / mL BSA and 0.1% NaN ₃

USE

This fluorochrome-conjugated antibody permits the identification and numeration of cell populations expressing the Glycophorin A (CD235a) antigen present in human biological samples using flow cytometry.

PRINCIPLE

This test is based on the ability of specific monoclonal antibodies to bind to the antigenic determinants expressed by cells.

Specific staining of the cells is performed by incubating the sample with the IOtest reagent.

The flow cytometer measures light diffusion and the fluorescence of cells. It makes possible the delimitation of the population of interest within the electronic window defined on a histogram, which correlates the orthogonal diffusion of light (Side Scatter or SS) and the diffusion of narrow-angle light (Forward Scatter or FS). Other histograms combining two of the different parameters available on the cytometer, can be used as supports in the gating stage depending on the application chosen by the user.

The fluorescence of the delimited cells is analyzed in order to distinguish the positively-stained events from the unstained ones. The results are expressed as a percentage of positive events in relation to all the events acquired by the gating.

EXAMPLES OF CLINICAL APPLICATIONS

Glycophorin A is a sialoglycoprotein expressed on the surface of erythroblastic precursor cells (from the pro-erythroblast stage) of reticulocytes and mature red blood cells (1 – 3).

This reactive permits the characterisation and the counting of cells belonging to the erythrocytic line.

STORAGE AND STABILITY

The conjugated liquid forms must be kept at between 2 and 8°C and protected from light, before and after the vial has been opened.

Stability of closed vial: see expiry date on vial.

Stability of opened vial: the reagent is stable for 90 days.

PRECAUTIONS

- Do not use the reagent beyond the expiry date.
- Do not freeze.
- Let it come to room temperature (18 – 25°C) before use.
- Minimize exposure to light.
- Avoid microbial contamination of the reagents, or false results may occur.
- Antibody solutions containing sodium azide (NaN₃) should be handled with care. Do not take internally and avoid all contact with the skin, mucosa and eyes.

Moreover, in an acid medium, sodium azide can form the potentially dangerous hydrazoic acid. If it needs to be disposed of, it is recommended that the reagent be diluted in a large volume of water before pouring it into the drainage system so as to avoid the accumulation of sodium azide in metal pipes and to prevent the risk of explosion.

- All blood samples must be considered as potentially infectious and must be handled with care (in particular: the wearing of protective gloves, gowns and goggles).
- Never pipette by mouth and avoid all contact of the samples with the skin, mucosa and eyes.
- Blood tubes and disposable material used for handling should be disposed of in ad hoc containers intended for incineration.

SAMPLES

Venous blood or bone marrow samples must be taken using sterile tubes containing an EDTA salt as the anticoagulant. The use of other anticoagulants is not recommended.

The samples should be kept at room temperature (18 – 25°C) and not shaken. The samples should be homogenized by gentle agitation prior to taking the test sample. The samples must be analyzed within 24 hours of taking them.

METHODOLOGY

NECESSARY MATERIAL NOT SUPPLIED

- Sampling tubes and material necessary for sampling.
- Automatic pipettes with disposable tips for 20, 100 and 500 µL.
- Plastic haemolysis tubes.
- Calibration beads: Flow-Set™ Fluorospheres (Ref.6607007).
- Red cell lysis reagent with washing stage after lysis. For example: VersaLyse (Ref. A09777).
- Leucocyte fixation reagent. For example : IOtest 3 Fixative Solution (Ref. A07800).
- Isotypic control: IOtest reagent. IgG1-PE (Ref. A07796).
- Buffer (PBS: 0.01 M sodium phosphate; 0.145 M sodium chloride; pH 7.2).
- Centrifuge.
- Automatic agitator (Vortex type).
- Flow cytometer.

PROCEDURE

NOTE: The procedure below is valid for standard applications. Sample and/or VersaLyse volumes for certain Beckman Coulter applications may be different. If such is the case, follow the instructions on the application's technical leaflet. For each sample analyzed, in addition to the test tube, one control tube is required in which the

cells are mixed in the presence of an isotypic control (Ref. A07796).

- Add 20 µL of specific IOtest conjugated antibody to each test tube, and 20 µL of the appropriate isotypic control to each control tube.
- Add 100 µL of the test sample to both tubes. Vortex the tubes gently.

ATTENTION : Too many red blood cells

(RBCs) in the sample can give rise to a lack of staining in the target leucocyte population. In this case, dilute the sample in PBS in order to obtain a concentration of approximately 1 x 10⁴ cells (RBCs + leucocytes) / µl then use 100 µl of this suspension, or isolate the mononuclear cells by means of a density gradient (Ficoll for example) and use 100 µl of this suspension the cellular concentration of which will have been adjusted to 1 x 10⁴ cells / µl.

- Incubate for 15 to 20 minutes at room temperature (18 – 25°C), protected from light.
- Then perform lysis of the red cells, if necessary, by following the recommendations of the lysis reagent used. As an example, if you wish to use VersaLyse (Ref. A09777), refer to the leaflet and follow preferably the procedure called "with concomitant fixation", which consists of adding 1 mL of the "Fix-and-Lyse" mixture prepared extemporaneously. Vortex immediately for one second and incubate for 10 minutes at room temperature, protected from light. If the sample does not contain red cells or if you want to keep the red cells, add 2 mL of PBS.
- Centrifuge for 5 minutes at 150 x g at room temperature.
- Remove the supernatant by aspiration.
- Resuspend the cell pellet using 3 mL of PBS.
- Repeat step 5.
- Remove the supernatant by aspiration and resuspend the cell pellet using:
 - 0.5 mL or 1 mL of PBS plus 0.1% of formaldehyde if the preparations are to be kept for more than 2 hours and less than 24 hours. (A 0.1% formaldehyde PBS can be obtained by diluting 12.5 µL of the IOtest 3 Fixative Solution (Ref. A07800) at its 10X concentration in 1 mL of PBS).
 - 0.5 mL or 1 mL of PBS without formaldehyde, if the preparations are to be analyzed within 2 hours.

NOTE: In all cases, keep the preparations between 2 and 8°C and protected from light.

PERFORMANCE

SPECIFICITY

The monoclonal antibody 11E4B-7-6 (KC16) reacts with the 27-39 terminal N amino acid sequence of Glycophorin A and does not recognize Glycophorin B (3). It was assigned to CD235a during the 7th HLDA workshop on Human Leucocyte Differentiation Antigens, held in Harrogate, England, in 2000 (WS Code : 70359, Section : Red Cells) (4).

LINEARITY

To test the linearity of staining of this reagent, a positive cell line (HEL) and a negative cell line (FRN 17.4.14.33) were mixed in different proportions with a constant final number of cells, so that the positive line/negative line ratio of the mixture ranged from 0 to 100%.

Aliquots were stained using the procedure described above and linear regression between the expected values and the observed values was calculated. The parameters of the equation of the linear regression may be used to determine the linearity as well as the range of measurement.

Specificity	Linear regression	Linearity (R ²)	Range (%)
Glycophorin A	Y = 0.97 X + 0.81	0.997	1 – 98

EXPECTED VALUES

The unlysed blood of healthy adults (to enable red blood cell staining) is 100% positive for Glycophorine A. No positive staining occurs after lysis of red blood cells, whether for lymphocytes, monocytes or granulocytes. Under these conditions the expected results for this specificity cannot be given.

INTRA-LABORATORY REPRODUCIBILITY

On the same day and using the same cytometer, 12 measurements of the percentage of positive cells (mixture of approximately 30% of positive cells from the HEL cell line and

negative cells from the FRN 17.4.14.33 line) were carried out. The results obtained are summarized in the following table:

Positive Target	Number	Mean (%)	SD	CV (%)
HEL line	12	26.5	0.7	2.5

INTER-LABORATORY REPRODUCIBILITY

On the same day for the same positive target (mixture of approximately 30% of positive cells from the HEL cell line and negative cells from the FRN 17.4.14.33 line), 12 measurements of the percentage of positive cells were carried out by two technicians and the preparations analyzed using two different cytometers. The results obtained are summarized in the following tables:

Cytometer n° 1:

Positive Target	Number	Mean (%)	SD	CV (%)
HEL line	12	26.5	0.7	2.5

Cytometer n° 2:

Positive Target	Number	Mean (%)	SD	CV (%)
HEL line	12	24.7	0.6	2.6

LIMITATIONS OF THE TECHNIQUE

1. Flow cytometry may produce false results if the cytometer has not been aligned perfectly, if fluorescence leaks have not been correctly compensated for and if the regions have not been carefully positioned.
2. It is preferable to use a technique with washing as this reagent has not been optimized for "without washing" techniques.
3. Accurate and reproducible results will be obtained as long as the procedures used are in accordance with the technical insert

leaflet and compatible with good laboratory practices.

4. The conjugated antibody of this reagent is calibrated so as to offer the best specific signal/non-specific signal ratio. Therefore, it is important to adhere to the reagent volume/number of cells ratio in every test.
5. In the case of a hypercellularity, dilute the blood in PBS so as to obtain a value of approximately 5×10^9 cells/L.
6. In the event of red blood cell lysis, account should be taken of the fact that in certain diseases, such as severe renal failure or in haemoglobinopathies, lysis may be slow, incomplete or even impossible. In this case, it is advisable to isolate mononucleated cells using a density gradient (Ficoll, for example) prior to staining.

MISCELLANEOUS

See the Appendix for references.

TRADEMARKS

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MANUFACTURED BY:

IMMUNOTECH S.A.
a Beckman Coulter Company
130 avenue de Lattre de Tassigny
B.P. 177 – 13276 Marseille Cedex 9
France
Customer Services: (33) 4 91 17 27 27

www.beckmancoulter.com



APPENDIX TO REF A07792

REFERENCES

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3. Catimel, B., Wilson, K.M., Kemp, B.E., "Kinetics of the autologous red cell agglutination test", 1993, *J. Immunol. Methods*, 165, 183-192.
4. Van der Schoot, C.E., Baardman, R., Ligthart, P., de Jong, I., EG KR von dem Borne, A., de Haas, M., "Red Cell Section: Section Report", 2000, *Leucocyte Typing VII, White Cell Differentiation Antigens*, D. Masson, et al., Eds., Oxford University Press, 566-604.