IOTest®

CD56-PE

A07788
100 tests; 2 mL
20 µL / test

IOTest
Conjugated Antibody
IVD CE

ENGLISH

	Specifications
Specificity	CD56
Clone	N901 (NKH-1)
Hybridoma	NS1/1-Ag4 x Balb/c
Immunogen	Human chronic myeloid leukaemia cells
Immunoglobulin	IgG1
Species	Mouse
Source	Ascites
Purification	Protein A affinity chromatography
Fluorochrome	R Phycoerythrin (PE)
λ excitation	488 nm
Emission peak	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 CD56 lymphocyte populations 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 leucocytes.
Specific staining of the leucocytes is performed by incubating the sample with the IOTest reagent. The red cells are then removed by lysis and the leucocytes, which are unaffected by this process, are analyzed by flow cytometry.
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 aids 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 electronic gating.

EXAMPLES OF CLINICAL APPLICATIONS

The monoclonal antibody (mAb) N901 (or NKH-1) recognizes the CD56 antigen, which is expressed by the majority of NK (Natural Killer) cells in peripheral blood (1, 2) and by a sub-population of circulating ($CD3^+$) T cell lymphocytes (3).
NK cells are characterized by innate and specific cytotoxic activity, which takes place when the balance between inhibitory signals received at the level of their receptors, called KIRs (Killer Immunoglobulin-like Receptors), and the activating signals received at the level of their NCR (Natural Cytotoxicity) receptors is broken. Thus, NK cell KIRs, by recognizing a sufficient number of class I HLA molecules at the surface of the target cell, deliver a signal capable of inhibiting lysis. Conversely, if these HLA molecules are insufficient in number (the case of tumor cells or cells infected by a virus), then the lysis signal carried by NCRs is not inhibited, leading to lysis of the target cell (4).
In healthy individuals, more than 95 % of cells capable of inducing this innate and specific cytotoxic activity are found in the 15 % (approximately) of lymphocytes in peripheral blood which express the NKH-1 antigen.
This reagent enables cellular populations or sub-populations with NK and/or cytotoxic activity associated with the $CD56^+$ phenotype to be characterized and counted. It also permits the study of these populations in immune system diseases: AIDS and other immune deficiencies (2), autoimmune disorders, hypersensitivity, viral infections and the restoration of an immune response after bone marrow and/or organ transplantation (5). Finally, it can be useful for the phenotyping of malignant blood dyscrasias such as leukaemias and lymphomas (6 – 10).

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

1. Do not use the reagent beyond the expiry date.
2. Do not freeze.
3. Let it come to room temperature (18 – 25°C) before use.
4. Minimize exposure to light.
5. Avoid microbial contamination of the reagents, or false results may occur.
6. Antibody solutions containing sodium azide (NaN_3) 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.
7. 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).
8. Never pipette by mouth and avoid all contact of the samples with the skin, mucosa and eyes.
9. 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 venipuncture.

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 the isotypic control (Ref. A07796).

1. Add 20 µL of specific IOTest conjugated antibody to each test tube, and 20 µL of the isotypic control to each control tube.
2. Add 100 µL of the test sample to the 2 tubes. Vortex the tubes gently.
3. Incubate for 15 to 20 minutes at room temperature (18 – 25°C), protected from light.
4. 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, add 2 mL of PBS.
5. Centrifuge for 5 minutes at 150 x g at room temperature.
6. Remove the supernatant by aspiration.
7. Resuspend the cell pellet using 3 mL of PBS.
8. Repeat step 5.
9. 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

MaB N901 (NKH-1) was assigned to CD56 during the 4th HLDA Workshop on Human Leucocyte Differentiation Antigens, held in Vienna, Austria, in 1989 (WS Code: NK59, Section NK) (11).

LINEARITY

To test the linearity of staining of this reagent, an MO7E ($CD56^+$) line and peripheral blood diluted to 1/5000 ($CD56^-$ red blood cells) were mixed in different proportions and with a constant final number of cells, so that the positive line/red blood cells ratio of the mixture ranged from 0 to 100%.
Aliquots were stained using the procedure described above (without lysis) and linear regression between the expected values and the observed values was calculated.

Specificity	Linear regression	Linearity (R^2)
CD56	Y = 0.97 X + 0.22	0.999

EXPECTED VALUES

Each laboratory must compile a list of reference values based upon a group of healthy donors from the local population. This must be done by taking age, sex and ethnic group into account, as well as any other potential regional differences.
In our laboratories, the whole blood samples of 50 healthy adults were treated using the reagent described above. The $CD56^+$ lymphocyte percentage mean is given in the following table:

Lymphocytes	Number	Mean (%)	SD	CV (%)
$CD56^+$	50	17.2	6.3	36.6

INTRA-LABORATORY REPRODUCIBILITY

On the same day and using the same cytometer, 12 measurements of the percentage of $CD56^+$ lymphocytes were carried out on blood taken from the same donor. The results obtained are summarized in the following table:

Lymphocytes	Number	Mean (%)	SD	CV (%)
$CD56^+$	12	21.4	1.4	6.5

INTER-LABORATORY REPRODUCIBILITY

On the same day and on blood from the same donor, 12 measurements of the percentage of $CD56^+$ lymphocytes 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:

Lymphocytes	Number	Mean (%)	SD	CV (%)
$CD56^+$	12	21.4	1.4	6.5

Cytometer n° 2:

Lymphocytes	Number	Mean (%)	SD	CV (%)
$CD56^+$	12	19.6	0.6	3.2

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 RBC lysis technique with a washing step as this reagent has not been optimized for "no wash" lysis 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/sample volume ratio in every test.
5. In the case of a hyperleucocytosis, dilute the blood in PBS so as to obtain a value of approximately 5×10^9 leucocytes/L.
6. In certain disease states, such as severe renal failure or haemoglobinopathies, lysis of red cells may be slow, incomplete or even impossible. In this case, it is recommended to isolate mononucleated cells using a density gradient (Ficoll, for example) prior to staining.

MISCELLANEOUS

See the Appendix for examples and references.

TRADEMARKS

The Beckman Coulter logo, COULTER, EPICS, EXPO, Flow-Set, IOTest, System II, VersaLyse, and XL are registered trademarks of Beckman Coulter Inc.

BD FACScan is a registered trademark of BD Biosciences and Company.

MANUFACTURED BY:

IMMUNOTECH SAS
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

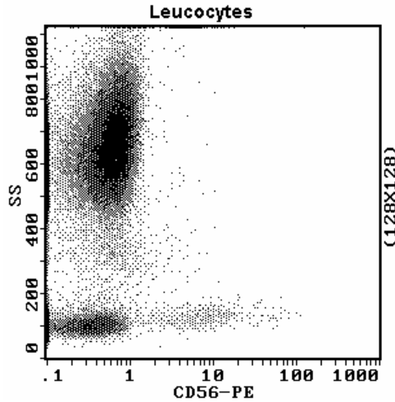
www.beckmancoulter.com

APPENDIX TO REF A07788

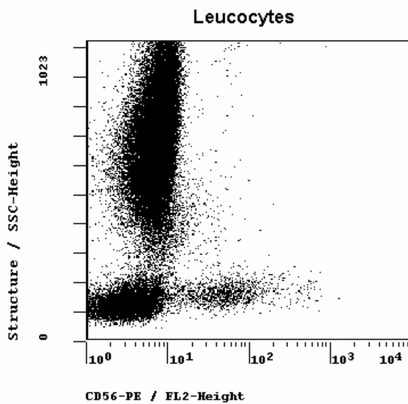
EXAMPLES

The histograms below are biparametric representations (Side Scatter versus Fluorescence Intensity) of lysed normal whole blood sample. Staining is with IOTest CD56-PE Conjugated Antibody (Ref. A07788). Gate is on leucocytes. The isotypic control (Ref. A07796) labelling is not shown.

Acquisition and analysis are performed with a COULTER® EPICS® XL™ flow cytometer equipped with System II™ software.



Acquisition is with a Becton Dickinson FACScan™ flow cytometer. Analysis is with EXPO™ software.



REFERENCES

1. Griffin, J.D., Hercend, T., Beveridge, R., Schlossman, S.F., "Characterization of an antigen expressed by human natural killer cells", 1983, J. Immunol., 130, 2947-2951.
2. Lanier, L.L., Le, A.M., Civin, C.I., Loken, M.R., Phillips, J.H., "The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes", 1986, J. Immunol., 136, 4480-4486.
3. Hercend, T., Griffin, J.D., Bensussan, A., Schmidt, R.E., Edson, M.A., Brennan, A., Murray, C., Daley, J.F., Schlossman, S.F., Ritz, J., "Generation of monoclonal antibodies to a human natural killer clone: Characterization of two natural killer-associated antigens, NKH1A and NKH2, expressed on subsets of large granular lymphocytes", 1985, J. Clin. Invest., 75, 932-943.
4. Whiteside, T.L., Herberman, R.B., "The role of natural killer cells in immune surveillance of cancer", 1995, Curr. Opin. Immunol., 7, 704-710.
5. Lanier, L.L., "The role of natural killer cells in transplantation", 1995, Curr. Opin. Immunol., 7, 626-631.
6. Robertson, M.J., Ritz, J., "Biological and clinical relevance of human natural killer cells", 1990, Blood,12, 76, 2421-2438.
7. Stewart, C.C., Behm, F.G., Carey, J.L., Cornbleet, J., Duque, R.E., Hudnall, S.D., Hurtubise, P.E., Loken, M., Tubbs, R.R., Wormsley, S., "U.S. Canadian consensus recommendations on the immunophenotypic analysis of hematologic neoplasia by flow cytometry: Selection of antibody combinations", 1997, Cytometry, 30, 231-235.
8. Rothe, G., Schmitz, G. Adorf, D., Barlage, S., Gramatzki, M., Hanenberg,H., Höffkes H.G., Janossy, G., Knüchel, R., Ludwig, W.D., Nebe, T., Nerl, C., Orfao, A., Serke, S., Sonnen, R., Tichelli, A., Wörmann, B., "Consensus protocol for the flow cytometric immunophenotyping of hematopoietic malignancies", 1996, Leukemia, 10, 877-895.
9. Rosenberg, S.A., "Classification of lymphoid neoplasms", 1994, Blood, 5, 84, 1359-1360.
10. Rothe, G., Schmitz, G., Adorf, D., Barlage, S., Gramatzki, M., Höffkes, H.G., Janossy, G., Knüchel, R., Ludwig, W.D., Nebe, T, Nerl, C., Orfao, A., Serke, S., Sonnen, R., Tichelli, A., Wörmann, B., "Consensus protocol for the flow cytometric immunophenotyping of hematopoietic malignancies", 1996, Leukemia, 10, 877-895.
11. Schubert, J., Lanier, L.L., Schmidt, R.E., "Cluster report: CD56", 1989, Leucocyte Typing IV, White Cell Differentiation Antigens. W. Knapp, et al., Eds., Oxford University Press, 699-702.