



NK Cytolytic Competence Assay (Human CD16/Perforin) Flow Cytometry Kit

Package Insert

Please read entire package insert completely
before using the product.

Not for resale

Catalog Number: 11-1002
Size: 25 Tests

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Part Number: 12-1006.0

Table of Contents

I. SUMMARY AND EXPLANATION	3
A. Intended Use	3
B. Principle of the Assay.....	3
II. WARNINGS AND PRECAUTIONS.....	3
A. Warnings	3
B. Precautions.....	4
III. COMPONENTS AND STORAGE.....	4
A. Kit Components.....	4
B. Required Materials (Not Supplied).....	4
C. Kit Storage	4
D. Sample Collection.....	5
E. Sample Transport (If sample needs to be transported)	5
IV. INSTRUCTIONS FOR USE.....	6
A. Time Required for Performing Assay.....	6
B. Reagent Preparation	6
C. Sample Preparation.....	6
D. Assay Procedure.....	6
E. Procedural Notes	7
F. Calculation of Results	9
V. TECHNICAL INFORMATION.....	9
A. Warranty.....	9
B. Technical Service.....	9
VI. BIBLIOGRAPHY.....	10

I. SUMMARY AND EXPLANATION

A. Intended Use

The intended use for the MSDx NK-Perforin immunophenotyping kit is the identification and enumeration of CD16 positive human lymphocytes (NK cells) that express Perforin as an aid to determination of immune status.

B. Principle of the Assay

Washed peripheral blood mononuclear cells (PBMCs) are incubated with phycoerythrin (PE) labeled monoclonal antibody to human CD16 antigen. Unbound antibody is washed off by centrifugation; the cells are then permeabilized and incubated with a fluorescein (FITC) labeled monoclonal antibody to human Perforin. The cells are then washed and fixed and then analyzed with a flow cytometer gated on lymphocytes.

II. WARNINGS AND PRECAUTIONS

A. Warnings

1. For Research Use Only. Not for Use in Diagnostic Procedures.
2. All specimens and materials with which they come into contact are considered biohazards, and should be handled as if capable of transmitting infection.
3. Follow proper precautions in accordance with federal, state and local regulations when disposing of all materials.
4. Never pipette by mouth. Avoid specimen contact with skin and mucous membranes.
5. Each antibody reagent contains sodium azide as a preservative. Care should be taken to avoid microbial contamination, which can cause erroneous results.
6. Sodium azide is harmful if swallowed. If swallowed, seek medical advice immediately and show the container or label.
7. Keep out of reach of children.
8. Keep away from food, drink and animal feed.
9. Wear suitable protective clothing.
10. Contact with acids liberates very toxic gas. Azide compounds should be flushed with large volumes of water during disposal to avoid deposits in lead or copper plumbing where explosive conditions can develop.

B. Precautions

1. This kit is intended for use by professionals who have the appropriate training for safe use and in accordance with all relevant regulations.
2. Use in accordance with BL2 safety precautions and all blood-borne pathogen regulations.
3. Dispose of materials in accordance with biohazard regulations.

III. COMPONENTS AND STORAGE

A. Kit Components

#	Component Description	Part #
01	Anti-Human Perforin FITC	13-1003
02	Anti-Human CD16 PE	13-1001
03	IgG2b FITC Negative Control (sub-class matched)	13-1004
04	IgG1 PE Negative Control (sub-class matched)	13-1002
05	Package Insert	12-1006

B. Required Materials (Not Supplied)

#	Material Description
01	Flow Cytometer capable of 2 color analyses
02	20, 100 and 1000 uL pipette with tips
03	Bench top centrifuge
04	Phosphate Buffered Saline, pH 7.4
05	IntraStain kit* (Dako Inc.)

*** This kit has been optimized using this permeabilization kit. Other permeabilization reagents may not give comparable results.**

C. Kit Storage

Store the kit at 2-8° C. Do not use the kit or any components after the expiration date on the packaging.

D. Sample Collection

It is recommended that blood be collected into a 4-8 ml CPT tube (Becton Dickinson) for isolation of PBMCs according to manufacturer's instructions.

E. Sample Transport (If sample needs to be transported)

For consistent results, MSDx recommends that the blood be transported by overnight courier in a Styrofoam box with outer cover. Transport methods must follow all applicable regulations and safety measures. MSDx has found no difference in transportation with a cold pack or at ambient temperature.

NK Cytolytic Competence Assay: Transit Temperature

FedEx Overnight

SAMPLE	%CD16 ⁺		%CD16 ⁺ /Perforin ⁺	
	ICE	AMBIENT	ICE	AMBIENT
1	26.74	26.84	88.04	90.91
2	37.14	37.12	84.97	82.57
3	26.11	26.56	91.19	90.70

Paired t-test %CD16+

P value and statistical significance:

The two-tailed P value equals 0.3368

This difference is not considered statistically significant by conventional criteria.

Paired t-test %CD16+/Perforin+

P value and statistical significance:

The two-tailed P value equals 0.9969

This difference is not considered statistically significant by conventional criteria.

IV. INSTRUCTIONS FOR USE

A. Time Required for Performing Assay

One hour and 30 minutes; not including time taken for isolation of PBMCs (see sample preparation).

B. Reagent Preparation

Prepare phosphate buffered saline (PBS) in sufficient quantity for use throughout the assay

C. Sample Preparation

It is recommended that blood be collected into a CPT tube (Becton Dickinson) for isolation of PBMCs according to manufacturer instructions. However, PBMC isolation by standard manual ficoll gradient methods can also be used. Wash isolated PBMCs three times in PBS and re-suspend to a cell density of approximately 10^7 cells / mL

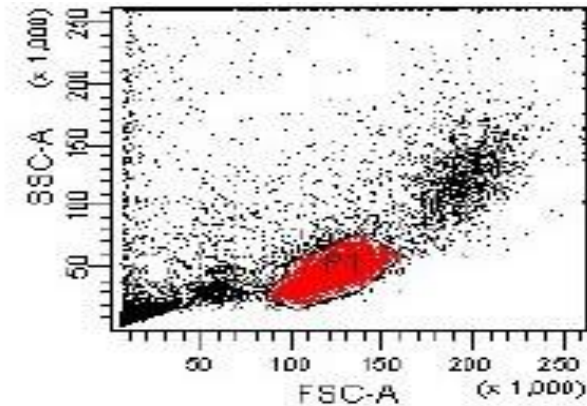
D. Assay Procedure

1. Transfer 50 to 60 ul (up to 1×10^6 cells) of the PBMC cell suspension to each of two FACS tubes labeled 1 and 2 (Control and Test).
2. To test tube 1 add 20ul of the IgG1 PE negative control. To test tube 2 add 20ul of the Anti-Human CD16 PE specific for the cell surface antigen to be stained (NK cells). Vortex gently and incubate in the dark for 20 minutes at room temperature.
3. Add 100 ul of Intrastain Fixation reagent A (Dako) to each tube. Mix gently to ensure all cells are in suspension.
4. Incubate at room temperature for 15 minutes.
5. Add 2 ml of PBS working solution to each test tube and mix gently.
6. Centrifuge at 250-300 x g for 5 minutes then aspirate the supernatant leaving approximately 50 ul of fluid. Mix thoroughly to ensure cells are in suspension.
7. Add 100 ul of Intrastain Permeabilization Reagent B (Dako), to tube 1 and 2. To test tube 1 add 20ul of IgG2b FITC negative control. To test tube 2 add 20 ul of Anti-Human Perforin FITC specific for the intracellular antigen to be stained. Mix thoroughly to ensure cells are in suspension.
8. Incubate in the dark for 20 minutes at room temperature.
9. Repeat steps 5 and 6.

10. Re-suspend the pellet in an appropriate fluid for flow cytometric analysis.
11. Analyze on a flow cytometer. If the samples are not analyzed immediately, they may be stored in the dark for up to 48 hours.

E. Procedural Notes

- a) **Setting the lymphogate:** Gate the lymphocytes by their size and granularity using forward scatter and side scatter parameters.



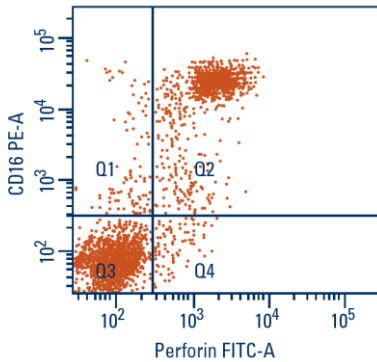
The lymphocyte gate may contain contamination by monocytes, particularly if the populations are not distinct by light scatter parameters. Consequently, it may be necessary to quality-control the gated population using the Leucogate kit from Becton Dickinson to gate on CD45⁺, CD14⁻ lymphocytes.

b) Spectral Compensation

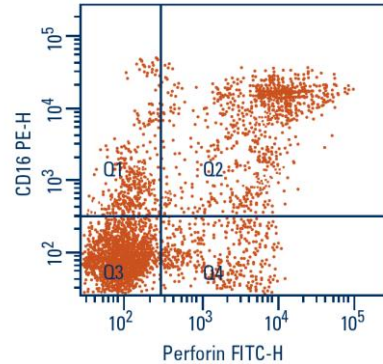
1. Perform instrument calibration/standardization procedures each day according to the established protocols of your laboratory.
2. Compensation setting and set up will vary when running normal fixed PBMC's versus those cells that have not been permeabilized.

3. We recommend setting compensation based on running permeabilized cells stained with CD 16PE and Perforin FITC individually (single stain). While monitoring 2-color dot plots, adjust compensation settings so that positively stained cells are directly in line with the unstained background cells and parallel with the appropriate axis. For example, with the CD16PE conjugated mAb stained cell population, adjust the FL1-%FL2 setting up or down so that the FL2 positive population is vertically aligned with the FL2 negative population (i.e., on the FL2 vs. FL1 dot plot). Then, with the Perforin FITC mAb stained cell population, adjust the FL2-%FL1 setting up or down so that the FL1 population is horizontally aligned with the FL1 negative population (i.e., on the FL2 vs. FL1 dot plot).
4. Compensation settings may need to be readjusted for each multi-color staining experiment.

V. Data Collection: Collect the data in list mode for analysis with appropriate software (e.g. Win MDI 2.9) <http://www.cyto.purdue.edu/flowcyt/software.htm>



Left Panel: Example of high percentage population of CD16⁺ Perforin⁺ lymphocytes $\left(\frac{Q2}{Q1+Q2}\right) \times 100 = \left(\frac{26.4}{1.8+26.4}\right) \times 100 = 98\%$



Right Panel: Example of low percentage population of CD16⁺ Perforin⁺ lymphocytes $\left(\frac{Q2}{Q1+Q2}\right) \times 100 = \left(\frac{16.2}{8.3+16.2}\right) \times 100 = 65\%$

A. Calculation of Results

Q1 = CD16⁺, Perforin⁻ lymphocytes.

Q2 = CD16⁺, Perforin⁺ lymphocytes.

Q3 = CD16⁻, Perforin⁻ lymphocytes.

Q4 = CD16⁻, Perforin⁺ lymphocytes (Cytotoxic T-cells).

$$\% \text{CD16}^+, \text{Perforin}^+ \text{ lymphocytes} = \left(\frac{Q2}{Q1+Q2} \right) \times 100$$

VI. TECHNICAL INFORMATION

A. Warranty

The product sold hereunder is warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, extending beyond the description on the label of the product. The sole liability of MSDx is limited to either product replacement or refund of the purchase price. MSDx is not liable for property damage, personal injury or economic loss caused by the product.

B. Assay Parameters

Intra-assay Variation: Ten replicates of a PBMC preparation from a healthy donor were assayed as described above with the following results:

Mean 91.6810; SD 0.4127; %CV = 0.45%

Inter-assay Variation: PBMCs from three healthy donors were assayed separately five times as described above with the following results.

Subject	Mean	SD	%CV
1	90.8820	0.6144	0.68
2	80.1980	0.9483	1.18
3	91.6980	0.5618	0.61

Inter-Laboratory Variation: Three CPT tubes of blood from three healthy subjects were drawn and one tube from each subject was sent by overnight courier to each of three geographically separate laboratories for analysis as described above with the following results:

Sample	X	Y	Z
mean	86.567	84.467	93.267
SD	0.95	4.827	2.65
CV (%)	1.1	5.71	2.841

Instrument: Comparable results were obtained using a BD FACScan™, ARIA I™ and an ARIA II™.

C. Technical Service/Customer Support

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VII. BIBLIOGRAPHY

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