

PN IM3487**25 tests****20 µL/test****CD16-FITC****CD56-PE****CD3-ECD**
IO Test[®] 3
 Conjugated Antibodies

For Research Use Only. Not For Use In Diagnostic Procedures.
REAGENT
 IO Test 3 Conjugated Antibodies – CD16-FITC / CD56-PE / CD3-ECD
 PN IM3487 – 25 tests – 20 µL/test

	CLONE 1	CLONE 2	CLONE 3
Specificity	CD16	CD56	CD3
Clone	3G8	N901 (NKH-1)	UCHT1
Hybridoma	SP2/0 x Balb/c	NS1/1-Ag4 x Balb/c	NS1 x Balb/c
Immunogen	Human neutrophils	Human chronic myeloid leukemia cells	Peripheral blood lymphocytes
Ig Chain	IgG1	IgG1	IgG1
Species	Mouse	Mouse	Mouse
Source	Ascites fluid	Ascites fluid	Ascites fluid
Purification	Ion exchange or affinity chromatography	Ion exchange or affinity chromatography	Ion exchange or affinity chromatography
Buffer	2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.		
Conjugation	Fluoresceine isothiocyanate (FITC)	PE (R-Phycoerythrin)	ECD™ (Phycoerythrin-Texas-Red®-X)

SPECIFICITY

The CD16 antigen is the low-affinity receptor for IgG (FcγRIII). The CD16 antigen exists in two different forms encoded by two different genes: FcγRIIIA (or III-2) and FcγRIIIB (or III-1). One is a transmembrane form (FcγRIIIA) expressed on NK cells, monocytes and macrophages. The other is a glycosylphosphatidylinositol (GPI)-anchored form (FcγRIIIB) only expressed on neutrophils (1, 2).

The 3G8 monoclonal antibody (mAb) binds to the FcγRIIIA as well as to the FcγRIIIB (strongly).

It has been assigned to the CD16 cluster of designation during the 4th International Workshop on Human Leucocyte Differentiation Antigens (HLDA) in Vienna, Austria, in 1989 (WS Code 409, Section NL) (3).

The CD56 antigen is known as the NCAM antigen (Neural Cell Adhesion Molecule) expressed on a subpopulation of peripheral blood large granular lymphocytes and on cells with natural killer (NK) activity. The N901 (NKH-1) mAb reacts with the majority of NK cells (4, 5). It also reacts with a minor subpopulation of CD3⁺ T cells that mediates reduced cytotoxic activity (6). This antibody does not react with monocytes, granulocytes, erythrocytes or B lymphocytes.

The N901 (NKH-1) mAb has been assigned to the CD56 cluster of differentiation during the 4th International HLDA Workshop in Vienna, Austria, in 1989 (WS Code: 9, Section NL) (7).

The CD3 antigen is a complex of 5 polypeptidic chains: γ, δ, ε, ζ and η associated with the T-cell receptor (TCR) complex (8). The CD3 antigen is expressed by mature T lymphocytes and by a subset of thymocytes (9).

The UCHT1 mAb reacts with the ε chain of the CD3 complex (10). It has been assigned to the CD3 cluster of differentiation at the 1st International HLDA Workshop in Paris, France, in 1982 (WS Code: 3, Section T) (11).

CONJUGATION

Fluorescein isothiocyanate (FITC) is conjugated at 3–8 moles of FITC per mole of Ig. Excitation wavelength: 488 nm
 Maximum emission wavelength: 525 nm
 Main emission color: Green

R-phycoerythrin (PE) is conjugated at 0.5–1.5 moles of PE per mole of Ig.

Excitation wavelength: 488 nm
 Maximum emission wavelength: 575 nm
 Main emission color: Orange-red

R-phycoerythrin covalently linked to Texas Red (PE-TxR or ECD) is conjugated at 0.5–1.5 moles of ECD per mole of Ig.

Excitation wavelength: 488 nm
 Maximum emission wavelength: 613 nm
 Main emission color: Red

APPLICATION

Multiparametric flow cytometry analysis of CD16, CD56, and CD3 antigen expression in hematopoietic neoplasia.

Characterization and differentiation between LGL T-cells leukemias (Large Granular Lymphocytic) (CD16⁺CD56⁺CD3⁺) and LGL NK-cells leukemias (CD16⁺CD56⁺CD3⁻) (12–15). Refer to complementary IO Test[®] 3 Conjugated Antibody reagent CD8-FITC/CD4-PE/CD3-ECD (PN IM3485) to confirm the T-Cell origin (CD8⁺CD4⁻CD3⁺) or NK origin (CD8^{possibly}⁺CD4⁻CD3⁻) of the studied neoplasia.

STATEMENT OF WARNINGS

1. This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes
4. Do not use antibody beyond the expiration date on the label.
5. Do not expose reagents to strong light during storage or incubation.
6. Avoid microbial contamination of reagents or incorrect results might occur.

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**BECKMAN
 COULTER™**

PN IM3487**25 tests****20 µL/test****CD16-FITC****CD56-PE****CD3-ECD****For Research Use Only. Not For Use In Diagnostic Procedures.****STORAGE CONDITIONS AND STABILITY**

Each reagent is stable up to the expiration date when stored at 2 – 8°C. Do not freeze. Minimize exposure to light.

REAGENT PREPARATION

No reconstitution is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 18 – 25°C prior to use.

PROCEDURE

This reagent is designed for flow cytometry.

Assay volume: 20 µL per 5 x 10⁵ cells in one test, or per 100 µL whole blood or bone marrow.

A wash is required to yield optimal results.

The use of IOTest 3 Lysing Solution (PN IM3514) and IOTest 3 Fixative Solution (PN IM3515) procedures are recommended to yield optimal results.

SELECTED RESEARCH REFERENCES

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MISCELLANEOUS

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