COULTER CLONE®
NKH-1

REF **6602705 - 100 tests**

PN 179057-A

BECKMAN COULTER™

ANALYTE SPECIFIC REAGENT
Analytical and performance characteristics are not established.

ANTIBODY SPECIFICITY
The antibody to the CD56 antigen identifies an isoform of the neural cell adhesion molecule (NCAM).[1] NCAM, a product of alternative splicing and heterogeneous glycosylation, has molecular weights ranging from 135 to 220 kD.[2-4] In hematopoietic cells, the 140 kD isoform is expressed exclusively on a subpopulation of lymphocytes that demonstrates natural killer activity.[2, 3, 5] Virtually all of these cells capable of mediating non-TCR mediated cytotoxicity in peripheral blood express CD56.[5, 6] This subpopulation consists of both natural killer cells (CD3-/CD56+) and a small subset of T cells (CD3+/ CD56+).[3, 4, 7] CD56 is not expressed on other T or B lymphocyte, monocyte, granulocyte, or erythrocyte populations.[7-9]

REAGENT
COULTER CLONE NKH-1
PN 6602705 - 100 tests (0.5 mL)

CLONE:	N901 (NKH-1) was derived from the hybridization of mouse NS-1 cells with spleen cells from BALB/c mice immunized with human chronic myeloid leukemia cells.[1]
Ig CHAIN:	Mouse IgG1 heavy and kappa light chains
SOURCE:	Conditioned media
PURIFICATION:	Affinity chromatography

REAGENT CONTENTS
The final concentration of nonantibody reagents in NKH-1 when reconstituted is 0.2% BSA, 0.01 M potassium phosphate, 0.15 M NaCl and 0.1% NaN_3.

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 reagent beyond the expiration date on the vial label.
5. Minimize exposure of reagent to light during storage or incubation.
6. Avoid microbial contamination of reagents or erroneous results may occur.
7. Use Good Laboratory Practices (GLP) when handling this reagent.
8. Harmful if swallowed.
9. After contact with skin, wash immediately with plenty of water.

STORAGE CONDITIONS AND STABILITY
Unreconstituted, lyophilized reagents are stable to the expiration date on the vial label when stored at 2-8°C. Do not freeze. Minimize exposure to light.

Reconstituted stock solutions of lyophilized reagent are stable as follows:
- 6 months when stored at 2-8°C or 0 to -20°C when reconstituted using the Reconstitution Procedure described in the REAGENT PREPARATION section. If all of a reconstituted reagent is not to be used within 6 months, follow the Freezing Procedure.
- 1 year when stored at -70°C using the Freezing Procedure.

FREEZING PROCEDURE
MATERIALS REQUIRED BUT NOT SUPPLIED
PBS - Phosphate Buffered Saline (pH=7.2) PN 6603369
PBS containing 2% heat-inactivated fetal or newborn calf serum (FCS). Dilute 2 mL of calf serum to 100 mL with PBS.

1. Dilute the reconstituted stock solution of the COULTER CLONE reagent with PBS containing 2% FCS prior to freezing as follows:

   Add 5 µL reconstituted stock solution (1 test*) to 100 µL PBS with 2% FCS**.
   *These may be frozen in multiple test volume aliquots.
   **This yields 2X of the concentration of the working solution.

2. Prior to use, allow the frozen aliquot to reach 20-25°C.
3. The frozen aliquot, at 2X the final concentration, must be further diluted to equal the total volume as calculated in the REAGENT PREPARATION section. Dilute each aliquot with the appropriate volume of PBS without 2% FCS and mix well.
4. Avoid repeated freeze/thaw cycles. This will denature the antibody protein.
5. Do not store in a self-defrosting freezer.

EVIDENCE OF DETERIORATION
Any change in the physical appearance of this reagent*, or any major variation in values obtained for control samples may indicate deterioration and the reagent should not be used. If the lyophilized material appears moist, do not use.

***Normal Appearance of Reagent**

Purified:	Lyophilized-white plug
	Reconstituted-clear, colorless liquid

REAGENT PREPARATION
Reconstitute the lyophilized COULTER CLONE NKH-1 reagent by adding 500 µL of distilled water to the vial. This is the stock solution. Centrifuge the stock solution at 20-25°C at 100,000 x g for 10 minutes to optimize staining results. Use this liquid reagent directly from the vial as the stock solution. The reagent working solution* is prepared as follows (volume listed is on a per test basis):

Add 5 µL stock solution to 195 µL PBS**.
*Diluted reagent working solution is good for day of preparation only.
**PBS - Phosphate Buffered Saline (pH=7.2).

Bring reagent to 20-25°C prior to use.

USAGE
This reagent is for use with standard fluorescence microscopy and/or flow cytometry methodologies.

The use of NKH-1 in this reagent is not intended for enumeration of NKH-1 cells in clinical diagnostic applications.

SELECTED RESEARCH REFERENCES

1. Knapp W, Dörken B, Gilks WR, Rieber EP, Schmidt RE, Stein H and von dem Borne AEGK, eds. 1989. Leucocyte Typing IV. White Cell Differentiation Antigens. Oxford, UK:Oxford University Press. pp. 536, 541, 699-702.
2. Schlossman SF, Boumsell L, Gilks W, Harlan JM, Kishimoto T, Morimoto C, Ritz J, Shaw S, Silverstein R, Springer T, Tedder TF and Todd RF, eds. 1995. Leucocyte Typing V. Oxford, UK:Oxford University Press. Volume 1. p 270. Volume 2. pp. 1398-1400.
3. Barclay AN, Birkeland ML, Brown MH, Beyers AD, Davis SJ, Somoza C, Williams AF, eds. 1993. The Leucocyte Antigen Facts Book. London:Academic Press. pp.106-109, 228-229.
4. Robertson MJ and Ritz J. 1990. Biology and clinical relevance of human natural killer cells. Blood 76:2421-2438.
5. Griffin JD, Hercend T, Beveridge R and Schlossman SF. 1983. Characterization of an antigen expressed by human natural killer cells. J Immunol 130:2947-2951.
6. Hercend T, Griffin JD, Bensussan A, Schmidt RE, Edson MA, Brennan A, Murray C, Daley JF, Schlossman SF and Ritz J. 1985. 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. J Clin Invest 75:932-943.
7. Schmidt RE, Murray C, Daley JF, Schlossman SF and Ritz J. 1986. A subset of natural killer cells in peripheral blood displays a mature T cell phenotype. J Exp Med 164:351-356.
8. Schmidt RE, Michon JM, Woronicz J, Schlossman SF, Reinherz EL and Ritz J. 1987. Enhancement of natural killer function through activation of the T11 E rosette receptor. J Clin Invest 79:305-308.
9. Caligiuri M, Murray C, Buchwald D, Levine H, Cheney P, Peterson D, Komaroff AL and Ritz J. 1987. Phenotypic and functional deficiency of natural killer cells in patients with chronic fatigue syndrome. J Immunol 139:3306-3313.

PRODUCT AVAILABILITY
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For additional information in the USA, call 800-526-7694. Outside the USA, contact your local Beckman Coulter Representative.

TRADEMARKS

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