COULTER CLONE®
KC56 (T-200)-FITC,
KC56 (T-200)-RD1

REF **6603838 - 100 tests**
REF **6603839 - 100 tests**

PN 4235871-F

BECKMAN COULTER®

	KC56 (T-200)-FITC	KC56 (T-200)-RD1
Specificity	CD45	CD45
Clone	DW124-5-2[8]	DW124-5-2[8]
Hybridoma	Sp2/0-AG14 x BALB/c	Sp2/0-AG14 x BALB/c
Immunogen	Derivative of the CEM cell line	Derivative of the CEM cell line
Ig Chain	IgG1	IgG1
Species	Mouse	Mouse
Source	Conditioned media	Conditioned media
Purification	Affinity chromatography	Affinity chromatography
Fluorescence	Excites at 468-509 nm / Emits at 504-541 nm	Excites at 486-580 nm / Emits at 568-590 nm
Conjugation	FITC (Fluorescein isothiocyanate)	RD1 (Phycoerythrin)
Molar Ratio	FITC/Protein: 5-10	RD1/Protein: 0.5-1.5

ANALYTE SPECIFIC REAGENT
Analytical and performance characteristics are not established.

ANTIBODY SPECIFICITY
KC56 recognizes members of the CD45 family of pan leukocyte antigens with molecular weights of 180, 190, 210 and 220 kD.[1-3] CD45 is also known as the leukocyte common antigen (LCA). It is expressed on every type of hematopoietic cell except mature erythrocytes and their immediate progenitors.[4,5] It has not been detected in differentiated nonhematopoietic tissue.[4-7]

REAGENTS
See table above.

REAGENT CONTENTS
The antibody concentration is 10.0 µg/test. The final concentration of nonantibody reagents in KC56 (T-200)-FITC when reconstituted is 0.2% gelatin, 0.01 M potassium phosphate, 0.15 M NaCl and 0.1% NaN_3.

The antibody concentration is 10.0 µg/test. The concentration of nonantibody reagents in 0.5 mL KC56 (T-200)-RD1 is 0.2% BSA, 0.01 M potassium phosphate, 0.15 M NaCl, 0.1% NaN_3 and stabilizers.

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 reagents beyond the expiration date on the vial labels.
5. Minimize exposure of reagents 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 these reagents.
8. Harmful if swallowed.
9. After contact with skin, wash immediately with plenty of water.

STORAGE CONDITIONS AND STABILITY
Liquid or 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.

The stock solution of reconstituted lyophilized reagent is 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 of reconstituted stock solution (1 test*) to 100 µL PBS with 2% FCS**.
   *These may be frozen in multiple test volume aliquots.
   **This yields 2X 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 these reagents,* or any major variation in values obtained for control samples may indicate deterioration and the reagents should not be used. If the lyophilized material appears moist, do not use.

*Normal Appearance of Reagents
FITC labeled: Lyophilized-white to yellow-orange plug
Reconstituted-clear, colorless to yellow-green liquid
RD1 labeled: Clear, colorless to pinkish liquid

REAGENT PREPARATION
Reconstitute the lyophilized COULTER CLONE KC56 (T-200)-FITC 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 to prepare the reagent working solution.

No reconstitution is necessary for COULTER CLONE KC56 (T-200)-RD1. This COULTER CLONE reagent is used directly from the vial to prepare the reagent working 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
These reagents are for use with standard flow cytometry and fluorescence microscopy (KC56 (T-200)-FITC) methodologies.

The use of KC56 (T-200)-FITC and/or KC56 (T-200)-RD1 is not intended for the enumeration of CD45 cells in clinical diagnostic applications.

SELECTED RESEARCH REFERENCES

1. McMichael AJ, Beverley PCL, Cobbold S, Crumpton MJ, Gilks W, Gotch FM, Hogg N, Horton M, Ling N, MacLennan ICM, Mason DY, Milstein C, Spiegelhalter D and Waldman H, eds. 1987. Leukocyte Typing III. Oxford University Press, Oxford, UK. pp.796-801, Appendix E.
2. Newman W, Targan SR and Fast LD. 1984. Immunobiological and immunochemical aspects of the T-200 family of glycoproteins. Mol Imm 21(11):1113-1121.
3. Fabre JW and Williams AF. 1977. Quantitative serological analysis of a rabbit anti-rat lymphocyte serum and preliminary biochemical characterisation of the major antigen recognised. Transplantation 23:4.
4. Coffman RL and Weissman IL. 1981. B220: A B cell-specific member of the T-200 glycoprotein family. Nature 289: 681-683.
5. Dalchau R and Fabre JW. 1980. Identification with a monoclonal antibody of a predominantly B lymphocyte-specific determinant of the human leukocyte common antigen. J Exp Med 153:753-765.
6. Omary MB, Trowbridge IS and Battifora HA. 1980. Human homologue of murine T-200 glycoprotein. J Exp Med 152: 842-852.
7. Dalchau R, Kirkley J and Fabre JW. 1981. Monoclonal antibody to a human leukocyte-specific membrane glycoprotein probably homologous to the leukocyte-common (L-C) antigen of the rat. Eur J Immunol 10:737-744.
8. Schlossman SF, Boumsell L, Gilks W, Harlan JM, Kishimoto T, Morimoto C, Ritz J, Shaw S, Silverstein

R, Springer R, Tedder TF and Todd RF, eds. 1995. Leukocyte Typing V. Oxford University Press, Oxford, UK.

PRODUCT AVAILABILITY

COULTER CLONE KC56 (T-200)-FITC
PN 6603838 - 100 tests (0.5 mL)
OR
COULTER CLONE KC56 (T-200)-RD1
PN 6603839 - 100 tests (0.5 mL)

RD1 is licensed under patent 4,520,110.

For additional information or if damaged product is received, call Beckman Coulter Customer Service at 800-526-7694 (USA or Canada) or contact your local Beckman Coulter Representative.

TRADEMARKS

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