COULTER CLONE® KC57-FITC, KC57-RD1

6604665 - 100 tests
6604667 - 100 tests

PN 4236216-E

BECKMAN COULTER®

	CLONE 1	CLONE 2
Specificity	HIV-1 proteins 55, 39, 33, & 24kD of core antigen	HIV-1 proteins 55, 39, 33, & 24kD of core antigen
Clone	FH190-1-1	FH190-1-1
Hybridoma	Sp2/0-AG14 X BALB/c	Sp2/0-AG14 X BALB/c
Immunogen	Purified HIV-$1_{8E5/LAV}$	Purified HIV-$1_{8E5/LAV}$
Ig Chain	IgG1	IgG1
Species	Mouse	Mouse
Source	Conditioned media	Conditioned media
Purification	Affinity chromatography	Affinity chromatography
Fluorescence	Excites 468-509 nm, Emits 504-541 nm	Excites 486-580 nm, Emits 568-590 nm
Conjugation	FITC (Fluorescein Isothiocyanate)	RD1 (Phycoerythrin)
Molar Ratio	FITC/Protein: 3-10	RD1/Protein: 0.5-1.5

MONOCLONAL ANTIBODY

For Research Use Only.
Not for use in diagnostic procedures.

ANTIBODY SPECIFICITY

The KC57 antibody identifies on Western blot the 55, 39, 33 and 24 kD proteins of the core antigens of the Human Immunodeficiency Virus Type 1 (HIV-1). The 55 kD protein is the precursor protein for the core antigen.[1] The 39 and 33 kD proteins are intermediate products and the 24 kD protein is the core protein.[2,3]

REAGENT

COULTER CLONE KC57-FITC
PN 6604665 - 100 tests (0.5 mL)
OR
COULTER CLONE KC57-RD1
PN 6604667 - 100 tests (0.5 mL)

CLONE: FH190-1-1 (KC57) was derived from the hybridization of mouse Sp2/0-AG14 myeloma cells with spleen cells from BALB/c mice immunized with purified HIV-$1_{8E5/LAV}$.

Ig CHAIN: Mouse IgG1 heavy chain and kappa light chains

SOURCE: Conditioned media

PURIFICATION: Affinity chromatography

CONJUGATION: KC57-FITC (Fluorescein isothiocyanate)
KC57-RD1 (Phycoerythrin)

MOLAR RATIO: FITC/protein 3-10
RD1/protein 0.5-1.5

FLUORESCENCE:
FITC (Green) Excites at 468-509 nm
Emits at 504-541 nm
RD1 (Orange) Excites at 486-580 nm
Emits at 568-590 nm

REAGENT CONTENTS

The concentration of nonantibody reagents 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 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 reagent 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

This reagent is stable to the expiration date on the vial label when stored at 2-8°C. Do not freeze. Minimize exposure to light.

EVIDENCE OF DETERIORATION

Any change in the physical appearance of this reagent (FITC labeled = clear colorless to yellowish-green liquid; RD1 labeled = clear colorless to pink liquid) or any major variation in values obtained for control samples may indicate deterioration and the reagent should not be used.

PREPARATION OF FICOLL-PREPARED CELLS OR TISSUE CULTURE CELLS FOR ANTIBODY STAINING

COULTER CLONE KC57 monoclonal antibody should be used for cytoplasmic staining of p24 antigen in infected cell lines or Ficoll-prepared peripheral blood lymphocytes (PBLs). Various permeabilization procedures exist and each laboratory should optimize a procedure that works best for their studies. The following is a suggested method for staining cells.

NOTE: The use of methanol in this procedure destroys surface antigens. If dual staining of cytoplasmic and surface antigens is desired, alternate permeabilization reagents must be used.

MATERIALS REQUIRED BUT NOT SUPPLIED

Lysophosphatidyl choline (egg yolk, lysolecithin)
Absolute methanol
Nonidet P-40 (NP-40)
Paraformaldehyde
Phosphate buffered saline (PBS)

REAGENT PREPARATION

1. Prepare 20 µg/mL lysolecithin in 1% paraformaldehyde as follows:
   Dissolve 10 g of paraformaldehyde in 100 mL of 1N NaOH. Bring the total volume to 800 mL with PBS. Titrate the pH to 7.2 +/-0.1 using 4N HCl. Bring the total volume to 1 liter with PBS. Dissolve 10 mg lysolecithin in 500 mL of the 1% paraformaldehyde solution. Mix and store at 2-8ºC.
2. 100% absolute methanol (requires no preparation). Store at -10 to -20ºC.
3. Prepare 0.1% NP-40 as follows: Add 0.5 mL NP-40 to 499.5 mL of PBS. Mix and store at 2-8ºC.
4. The above reagent solutions have a minimum shelf life of 6 months, if stored as indicated.

PROCEDURE

NOTE: Bring monoclonal antibody reagent to room temperature prior to use.

1. Pellet 10^6 (Ficoll-prepared PBLs or cell line) per tube.

   NOTE: Lysolecithin must be brought to room temperature before use.

2. Fix by adding 1 mL of 20 µg/mL lysolecithin in 1% paraformaldehyde.
3. Vortex. Incubate for 2 minutes at room temperature.
4. Centrifuge for 5 minutes at 4ºC at 500 x g. Decant or aspirate supernatant. Vortex.
5. Add 2 mL of cold absolute methanol (-10 to -20ºC).
6. Vortex. Incubate on ice for 15 minutes.
7. Centrifuge for 5 minutes at 4ºC at 500 x g. Decant or aspirate supernatant. Vortex.
8. Add 1 mL of 0.1% NP-40 (2-8ºC).
9. Vortex vigorously. Incubate on ice for 5 minutes.
10. Centrifuge for 5 minutes at 4ºC at 500 x g. Decant or aspirate supernatant. Vortex.
11. Add 5 µL monoclonal antibody or isotype control and 195 µL PBS to cells.
12. Vortex. Incubate for 15 minutes at room temperature.
13. Add 1 mL of PBS. Centrifuge for 5 minutes at 4ºC at 500 x g. Decant or aspirate supernatant. Vortex.
14. Dilute sample with 1 mL of PBS and analyze on a flow cytometer.

SELECTED RESEARCH REFERENCES

1. Robey WG, Safai B, Oroszlan S, Arthur LO, Gonda MA, Gallo RC and Fischinger PJ. 1985. Characterization of envelope and core structural gene products of HTLV-III with sera from AIDS patients. Science 228:593-595.

2. Chassagne J, Verrele P, Dionet C, Clavel F, Barre-Sinoussi F, Chermann JC, Montagnier L, Gluckman JC and Klatzmann D. 1986. A monoclonal antibody against gag precursor: Use for viral protein analysis and antigenic expression in infected cells. J Immunol 136:1442-1445.
3. Schupbach J, Popovic M, Gilden RV, Gonda MA, Sargadharan MG and Gallo RC. 1984. Serologic analysis of a subgroup of human T-lymphotropic retroviruses (HTLV-III) associated with AIDS. Science 224:503-505.

PRODUCT AVAILABILITY

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RD1 is licensed under patent 4,520,110.

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

TRADEMARKS

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