COULTER CLONE®
4B4,
4B4-FITC,
4B4-RD1

REF **6603113 - 100 tests**
REF **6603109 - 100 tests**
REF **6603177 - 100 tests**

PN 4235597-G

	4B4	4B4-FITC	4B4-RD1
Specificity	CD29	CD29	CD29
Clone	4B4LDC9LDH8	4B4LDC9LDH8	4B4LDC9LDH8
Hybridoma	NS-1 x BALB/c	NS-1 x BALB/c	NS-1 x BALB/c
Immunogen	T lymphocyte line derived from *Saguinus oedipus*	T lymphocyte line derived from *Saguinus oedipus*	T lymphocyte line derived from *Saguinus oedipus*
Ig Chain	IgG1	IgG1	IgG1
Species	Mouse	Mouse	Mouse
Source	Ascites fluid	Ascites fluid	Ascites fluid
Purification	Affinity chromatography	Affinity chromatography	Affinity chromatography
Fluorescence	Non Applicable	Excites at 468-509 nm / Emits at 504-541 nm	Excites at 486-580 nm / Emits at 568-590 nm
Conjugation	Non Applicable	FITC (Fluorescein Isothiocyanate)	RD1 (Phycoerythrin)
Molar Ratio	Non Applicable	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 4B4 antibody recognizes the CD29 antigen, which has a molecular weight of 134 kD. This antigen is the β1 integrin and is the common β subunit of the VLA family of adhesion molecules.[1-4] It appears on approximately 41% of unfractionated human T lymphocytes, 41% of CD4+ lymphocytes, 43% of CD8+ lymphocytes, 5-30% of B lymphocytes and over 30% of null cells, macrophages and thymic lymphocytes.[2] CD29 is not present on granulocytes.[1]

The 4B4 monoclonal antibody identifies the helper/inducer (CD4+/CD29+) subpopulation of CD4 lymphocytes.[2]

REAGENT

See table above.

REAGENT CONTENTS

The antibody concentration is 5.0 µg/test (4B4) and 10.0 µg/test (4B4-FITC). The final concentration of nonantibody reagents when reconstituted is 0.2% BSA (4B4) or 0.2% gelatin (4B4-FITC), 0.01 M potassium phosphate, 0.15 M NaCl and 0.1% NaN_3.

The antibody concentration is 2.5 µg/test (4B4-RD1). The concentration of nonantibody reagents in 4B4-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 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

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

Reconstituted stock solution of 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 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 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 Reagents**

Purified:	Lyophilized-white plug Reconstituted-clear, colorless liquid
FITC labeled:	Lyophilized-white to yellow-orange plug Reconstituted-clear, colorless to yellow-green liquid
RD1 labeled:	Clear, pink to red liquid

REAGENT PREPARATION

Reconstitute the lyophilized COULTER CLONE 4B4 or 4B4-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. 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).

No preparation is necessary for COULTER CLONE 4B4-RD1. This COULTER CLONE reagent is used directly from the vial.

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

USAGE

This reagent is for use with standard fluorescence microscopy (4B4 and 4B4-FITC) and/or flow cytometry methodologies.

The use of 4B4, 4B4-FITC or 4B4-RD1 in this reagent is not intended for enumeration of CD29 cells in clinical diagnostic applications.

SELECTED RESEARCH REFERENCES

1. McMichael AJ, Beverly 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.
2. Morimoto C, Letvin NL, Boyd AW, Hagan M, Brown HM, Kornacki MM and Schlossman SF. 1985. The isolation and characterization of the human helper inducer T cell subset. J Immunol 134:3762-3769.
3. Knapp W, Dorken B, Gilks WR, Rieber EP, Schmidt RE, Stein H and Kr. von dem Burne AEG, eds. 1989. Leukocyte Typing IV. Oxford University Press, Oxford, UK.
4. Matsuyama T, Yamada A, Kay J, Yamada KM, Akiyama SK, Schlossman SF and Morimoto C. 1989. Activation of CD4 cells by fibronectin and anti-CD3 antibody: A synergistic effect mediated by the VLA-5 fibronectin receptor complex. J Exp Med 1170:1133-1148.

PRODUCT AVAILABILITY

COULTER CLONE 4B4
PN 6603113 - 100 tests (0.5 mL)
OR
COULTER CLONE 4B4-FITC
PN 6603109 - 100 tests (0.5 mL)
OR
COULTER CLONE 4B4-RD1
PN 6603177 - 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.

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