COULTER CLONE®
2H4-FITC,
2H4-RD1

REF **6603117 - 100 tests**
REF **6603181 - 100 tests**

PN 4235790-F

	2H4-FITC	2H4-RD1
Specificity	CD45RA	CD45RA
Clone	2H4LDH11LDB9	2H4LDH11LDB9
Hybridoma	NS-1 x BALB/c	NS-1 x BALB/c
Immunogen	T lymphocyte line derived from *Aotus trivirgatus* (Owl monkey).[6,7]	T lymphocyte line derived from *Aotus trivirgatus* (Owl monkey).[6,7]
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
Scatter Detection	Forward and/or side	Forward and/or side

MONOCLONAL ANTIBODY

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

ANTIBODY SPECIFICITY

The 2H4 antibody recognizes the CD45RA isoforms of the CD45 family of leukocyte antigens.[1,2] These isoforms contain the sequence encoded by exon A and have molecular weights of 220 and 200 kD.[2] The proteins are expressed on the surface of a subset of T cells, on B cells and weakly on monocytes.[3] CD45RA is highly expressed on CD4+ T cells which are designated as suppressor/ inducer cells and which are functionally naive.[4,5]

REAGENT

See table above.

REAGENT CONTENTS

The antibody concentration in 2H4-FITC is 5.0 µg/test. The final concentration of nonantibody reagents when reconstituted is 0.2% gelatin (2H4-FITC), 0.01 M potassium phosphate, 0.15 M NaCl and 0.1% NaN_3.

The antibody concentration in 2H4-RD1 is 2.5 µg/test. The concentration of nonantibody reagents in 0.5 mL 2H4-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.

Reconstituted stock solution 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:
2. 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.
3. Prior to use, allow the frozen aliquot to reach 20-25°C.
4. 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.
5. Avoid repeated freeze/thaw cycles. This will denature the antibody protein.
6. 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, pink to red liquid

REAGENT PREPARATION

Reconstitute the lyophilized COULTER CLONE 2H4-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 2H4-RD1. This COULTER CLONE reagent is used directly from the vial.

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

USAGE

These reagents are for use with standard flow cytometry and/or fluorescence microscopy (2H4-FITC) methodologies.

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, UK:Oxford University Press.
2. Streuli M, Matsuyama T, Morimoto C, Schlossman SF and Saito H. 1987. Identification of the sequence required for expression of the 2H4 epitope on the human leukocyte common antigens. J Exp Med 166:1567-1572.
3. Thomas A. 1989. The leucocyte common antigen family. Ann Rev Immunol 7:339-369.
4. Morimoto C, Matsuyama T, Rudd C, Forsgren A, Letvin N and Schlossman SF. 1988. Role of the 2H4 molecule in the activation of suppressor inducer function. Eur J Immunol 18:731-737.
5. Merkenschlager M, Terry L, Edwards R and Beverley P. 1988. Limiting dilution analysis of proliferative responses in human lymphocyte populations defined by the monoclonal antibody UCHL1: implications for differential CD45 expression in T cell memory formation. Eur J Immunol 18:1653-1661.
6. Morimoto C, Letvin NL, Distaso JA, Aldrich WR and Schlossman SF. 1985. The isolation and characterization of the human suppressor inducer T cell subset. J Immunol 134:1508-1515.
7. Morimoto C, Letvin NL, Distaso JA, Brown HM and Schlossman SF. 1986. The cellular basis for the induction of antigen-specific T8-suppressor cells. Eur J Immunol 16:198-204.

PRODUCT AVAILABILITY

COULTER CLONE 2H4-FITC
PN 6603117 - 100 tests (0.5 mL)
OR
COULTER CLONE 2H4-RD1
PN 6603181 - 100 tests (0.5 mL)

RD1 is licensed under patent 4,520,110.
2H4 is licensed under patent 4,649,106.

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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