COULTER CLONE® B1

REF A83477 - 100 tests

PN A83483-AA

	B1
Specificity	CD20
Clone	H299
Hybridoma	P3/NS1/1-AG4-1 x BALB/c
Immunogen	Human B cell ascites Burkitt's lymphoma cells.[1]
Ig Chain	IgG2a[2]
Species	Mouse
Source	Mouse ascites fluid or conditioned cultured media
Purification	Ion Exchange Chromatography
Fluorescence	Non Applicable
Conjugation	Non Applicable
Molar Ratio	Non Applicable
Scatter Detection	Forward and/or side

ANALYTE SPECIFIC REAGENT
Analytical and performance characteristics are not established.

ANTIBODY SPECIFICITY
B1 (CD20) antigen is found on all B cells isolated from the peripheral blood, lymph node, spleen, tonsil, and bone marrow.[1] It is not found on normal T cells, monocytes, or granulocytes.[1,2] Unlike surface immunoglobulin which may bind via the Fc receptors of B cells, monocytes and subsets of T cells, and give a spurious result, the B1 antigen is an integral component of the B cell membrane.[1]

REAGENT
See table above.

REAGENT CONTENTS
The antibody concentration is 2.5 µg/test.

The final concentration of nonantibody reagents 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 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 lyophilized reagent is stable as follows:

- 6 months when stored at 2-8°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 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 B1 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 B1 in this reagent is not intended for enumeration of CD20 cells in clinical diagnostic applications.

SELECTED RESEARCH REFERENCES

1. Stashenko P, Nadler LM, Hardy R, and Schlossman SF: 1980. Characterization of a human B lymphocyte-specific antigen. J. Immunol. 125:1678-1685.
2. Leucocyte Typing II: 1986. Volume 2. Springer -Verlag NY, NY Reinherz EL, Haynes BF, Nadler LM, and Bernstein ID. editors. p. 8, 20.

PRODUCT AVAILABILITY
COULTER CLONE B1
REF A83477 - 100 tests (0.5 mL)

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