MODIFICATIONS IN CD4/CD8 RATIOS AND CD4+ AND CD8+ LYMPHOCYTE PERCENTAGES HAVE BEEN FOUND IN ACTIVE/INACTIVE SLE PATIENTS WITH MULTI-SYSTEM DISEASE BUT NO RENAL DISEASE. 13,14 HIGH CD4/CD8 RATIOS BUT DECREASED PERCENTAGES OF CD8+ LYMPHOCYTES HAVE ALSO BEEN DOCUMENTED IN SIMILAR ACTIVE SLE PATIENTS. 15 FURTHER, HIGH CD4+ AND LOW CD8+ LYMPHOCYTE PERCENTAGES HAVE BEEN MEASURED IN ACTIVE SLE PATIENTS WITH CENTRAL NERVOUS SYSTEM DISEASE BUT NO RENAL DISEASE. 13,14 IN CONTRAST, LOW CD4/CD8 RATIOS BUT INCREASED PERCENTAGES OF CD8+LYMPHOCYTES HAVE BEEN NOTED IN SLE PATIENTS WITH WIDESPREAD MULTI-SYSTEM SLE WHICH OFTEN INCLUDES THE RENAL AND CENTRAL NERVOUS SYSTEMS. 13,15

HIGH CD4/CD8 RATIOS HAVE BEEN FOUND IN PATIENTS WITH THYMIC APLASIA. 7,15

DECREASED CD4+ AND INCREASED CD8+ LYMPHOCYTE PERCENTAGES WITHOUT SIGNIFICANT CHANGES IN CD4/CD8 RATIOS HAVE BEEN OBSERVED IN PATIENTS WITH STABLE RENAL ALLOGRAFT FUNCTION AFTER TRANSPLANTATION. 20,21

LOW CD4/CD8 RATIOS AND DECREASED PERCENTAGES OF CD4+ LYMPHOCYTES HAVE BEEN DOCUMENTED IN PATIENTS DURING PHENOTYPIC RECONSTITUTION FOLLOWING PURGED AUTOGOUS BONE MARROW TRANSPLANTATION. 21

PRINCIPLES OF TEST

This test depends on the ability of a monoclonal antibody to bind to the surface of cells expressing discrete antigenic determinants. CYTO-STAT/COULTER CLONE CD8-ECD or IOTest CD8-PC7 is a murine monoclonal antibody specific for a cell surface antigen. Specific cell staining is accomplished by incubating whole blood with the CYTO-STAT/COULTER CLONE or IOTest reagent. Red blood cells are lysed and the remaining white blood cells are analyzed by flow cytometry using lymphocyte gates only. The percentage of positively-stained lymphocytes is determined for each sample. A duplicate whole blood sample stained with CYTO-STAT/COULTER CLONE MsIgG1-ECD or IOTest IgG1-PC7 isotypic control is used to assess nonspecific background fluorescence. (Label of isotypic control must correspond to label of monoclonal antibody.)

REAGENTS

See table above.

REAGENT CONTENTS

The antibody concentration in the CYTO-STAT/COULTER CLONE is 0.5 µg/test. Contact Beckman Coulter Customer Service to obtain the antibody concentration in the IOTest reagent.

The final concentration of nonantibody reagents in the CYTO-STAT/COULTER CLONE in 0.5 mL (1 vial) and IOTest 1.0 mL (1 vial) antibodies is 0.2% BSA, 0.01 M potassium phosphate, 0.15 M NaCl, 0.1% NaN3, and stabilizers.

STATEMENT OF WARNINGS

1. This reagent contains sodium azide. Sodium azide under acidic 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. Do not use antibody beyond the expiration date on label.

3. Samples and all material coming in contact with them should be handled as if capable of transmitting infection, and disposed of with proper precautions.

4. Never pipet by mouth and avoid contact of samples with skin and mucous membranes.

5. Minimize exposure of reagents to bright light during storage or incubation.

6. Incubation or centrifuge times or temperatures other than those specified may give erroneous results.

7. Avoid microbial contamination of reagents or erroneous results may occur.

8. If skin or eye contact occurs, wash immediately with plenty of water.

9. When acquiring without automated analysis and using FC 500 Flow Cytometry Systems with CXP Software ensure that "Events" is set to 100% in all dot plot displays.

10. Use Good Laboratory Practice (GLP) when handling reagent.

11. Review all histograms before reporting results.

REAGENT PREPARATION

None. The CYTO-STAT/COULTER CLONE and IOTest monoclonal antibody reagents are used directly from the...
vial with no dilution or centrifugation necessary. All reagents should be brought to 20-25°C prior to use.

**STORAGE CONDITIONS**
Unopened reagent is stable to the expiration date on the vial when stored at 2-8°C. Opened vials are stable for 90 days when stored at 2-8°C. Return reagent to 2-8°C immediately after use. Avoid freezing and exposure to light.

**EVIDENCE OF DETERIORATION**
Any change in the physical appearance of the reagents*, or any major variation in values obtained for control samples may indicate deterioration and the reagents should not be used.

*Normal Appearance of Reagents
ECD - labeled: Clear pink to red liquid
PC7 - labeled: Clear magenta to purple liquid

**SPECIMEN COLLECTION AND PREPARATION**

**CAUTION:** The stability of blood samples is quite variable. For optimal results, start the assay within 6 hours of venipuncture. Unstained, anticoagulated blood should remain at 20-25°C until processing is begun. Do not refrigerate.

Collect a venous blood sample aseptically by venipuncture into a blood collection tube using an appropriate anticoagulant (EDTA is recommended). For detailed information on the collection of whole blood by venipuncture and interfering conditions, refer to "Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture (H3, Approved Edition)" published by the Clinical and Laboratory Standards Institute. For each test, 100 µL of whole blood is required. Collect a sufficient amount of blood (1 to 2 mL required per tube) to run the test, control and have autologous plasma for sample dilution, if necessary. A white blood cell count should be performed.

**PROCEDURE FOR IMMUNOFLUORESCENCE CELL SURFACE STAINING WITH CYTO-STAT/COUNTERCLONE OR IOTEST MONOCLONAL ANTIBODY**

**MATERIAL SUPPLIED**

CYTO-STAT/COUNTERCLONE CD8-ECD
Monoclonal Antibody,
PN 737665 - 50 tests (0.5 mL)
OR
IOTest CD8-PC7 Monoclonal Antibody,
PN 737661 - 100 tests (0.5 mL)

**MATERIALS REQUIRED BUT NOT SUPPLIED**

Erythrocyte Lytic Reagent (as appropriate):
CYTOMED IMMUNOPREP™ Reagent System for
CYTOMED Q-PREP™ Workstation,
PN 7546946 - 100 tests
Diluent (if necessary) Autologous plasma
OR
COUNTER IMMUNOPREP Reagent System for
COUNTER MULTI-Q-PREP™ or TQ-Prep™ Workstation,
PN 7546999 - 300 tests
Diluent (if necessary) Autologous plasma
Flow-Count™ Fluorospheres,
PN 7547053
(Original Reagent)
Isotopic Control MegG1-ECD
OR
IOTest IgG1-PC7 Isotopic Control,
PN 737682

**INSTRUMENT REQUIREMENTS**

Flow cytometer that provides excitation and measures emission of scatter and fluorescence as specified in the table on page 1 as applicable for your specific product. Users should refer to the manufacturer’s instrument manuals for specific instructions for setting PMT voltages and fluorescence compensation prior to analysis.

**PROCEDURE**

1. Optimal staining is achieved with white blood cell counts in the range of 3-10 x 10^3 cells/µL. White blood cell counts exceeding 10 x 10^3 cells/µL require dilution, and white blood cell counts below 3 x 10^3 cells/µL require centrifugation and resuspension, to achieve counts in the range of 3-10 x 10^3 cells/µL. Autologous plasma is the recommended diluent when using the COUNTER IMMUNOPREP Reagent System.

**Abnormal Samples**

a. High White Blood Cell Count (>10 x 10^3 cells/µL) should be diluted to achieve counts in the range of 3-10 x 10^3 cells/µL.

b. Low White Blood Cell Count (<3 x 10^3 cells/µL) - Buffyo Coat Procedure
1) Centrifuge blood at 20-25°C at 500 x g for 5 minutes.
2) Draw off buffy coat with a Pasteur pipet, collecting some red blood cells and some plasma to assure that all white blood cells are recovered.
3) Fully resuspend cells by mixing several times with a Pasteur pipet.
4) Determine cell concentration using a cell counter or hemocytometer.
5) Adjust cell concentration to 10 x 10^3 cells/µL with diluent. Add 100 µL to antibody and follow standard procedure.

2. The appropriate isotype control should be run with each sample. For each sample, label two 12 x 75 mm test tubes, one for the monoclonal antibody and the other for the isotype control. Add 100 µL of the venous blood sample to each test tube. Care must be taken to avoid contamination of the tops and sides of the test tubes with blood or incomplete lysis may occur.

3. Add 10 µL of the CYTO-STAT/COUNTERCLONE CD8-ECD reagent to the labeled tubes. Alternatively, add 10 µL of the IOTest CD8-PC7 reagent or IOTest IgG1-PC7 isotypic control to the labeled test tubes.

4. Vortex gently. Incubate the reaction mixtures at 20-25°C for 10-12 minutes if using the COUNTER IMMUNOPREP Reagent System.

**IMPORTANT:** If blood droplets remain around the top of the test tube they must be removed or nonlysed red blood cells may contaminate the final sample and skew the results. A cotton tip applicator may be used for removal.
false decreased results due to unlysed red blood cells being counted as leukocytes.
8. Prolonged exposure of cells to lytic reagents may cause white blood cell destruction.
9. Results obtained with flow cytometry may be erroneous if the laser is misaligned or the gates are improperly set.
10. Due to an unacceptable variance among the different laboratory methods for determining absolute lymphocyte counts, an assessment of the accuracy of the method used is necessary.24

EXPECTED VALUES

Blood samples were collected from a population of apparently healthy males and females. This population included adults from a variety of races ranging in age. Samples were stained with CYTO-STAT/COULTER CLONE CD8-ECD or IOTest CD8-PC7 monoclonal antibody. Normal CD8+ cell values determined by flow cytometry (COULTER EPICS XL-MCL gated on lymphocytes) for whole blood are given in the following table. These are intended as representative values only. Each laboratory should establish its own expected values from the local population of normal donors.

WHOLE BLOOD

<table>
<thead>
<tr>
<th>CD8-ECD</th>
<th>CD8-PC7</th>
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<tbody>
<tr>
<td>CYTO-STAT/COULTER CLONE</td>
<td>IOTest</td>
</tr>
<tr>
<td>CD8-ECD %CD8+ Lymphocytes</td>
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</tr>
<tr>
<td>20</td>
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<td>48</td>
</tr>
<tr>
<td>28.9 ±6.9</td>
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PERFORMANCE CHARACTERISTICS

SPECIFICITY

The SFC121Thy2D3 (CD8) monoclonal binds to a nonpolymorphic domain of the MHC Class I molecules. SFC121Thy2D3 (CD8) was assigned to CD8 during the immunologic characterization of the human suppressor inducer T cell subset. J. Immunol. 16: 198-204.

LINEARITY

To test the linearity of staining for CD8-ECD and CD8-PC7, a positive control cell (CYTO-TROL Control Cells) was concentrated. The appropriate range of CD8-ECD positive cells, 9 to 9,123 cells, and the percent CD8+ cells were measured over this range. Aliquots were stained and the linear regression equation was calculated. The parameters of the equation of the linear regression may be used to determine the linearity as well as the range of measurement.

SPECIFICITY

The SFC121Thy2D3 (CD8) monoclonal binds to a nonpolymorphic domain of the MHC Class I molecules. SFC121Thy2D3 (CD8) was assigned to CD8 during the immunologic characterization of the human suppressor inducer T cell subset. J. Immunol. 16: 198-204.

CD8-PC7

Staining below is with CYTO-STAT/COULTER CLONE CD8-ECD Conjugated Monoclonal Antibody (PN 737659). Gate is on lymphocytes. A mouse PC7-conjugated IgG1 isotopic control (PN 737662) is shown.

Acquisition and analysis for CD8-PC7 are performed with a Cytomics FC 500 flow cytometer.

CD8-ECD

Staining below is with CYTO-STAT/COULTER CLONE CD8-ECD Conjugated Monoclonal Antibody (PN 737659). Gate is on lymphocytes. A mouse ECD-conjugated IgG1 monoclonal antibody. Normal CD8+ cell values determined by flow cytometry (COULTER EPICS XL-MCL gated on lymphocytes) for whole blood are given in the following table. These are intended as representative values only. Each laboratory should establish its own expected values from the local population of normal donors.

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**PRODUCT AVAILABILITY**

CYTO-STAT/COUlTER CLONE CD8-ECD
Monoclonal Antibody,
PN 737659 - 50 tests (0.5 mL)
IOTest CD8-PC7 Monoclonal Antibody,
PN 737661 - 100 tests (1.0 mL)

ECD is licensed under patents 4,542,104 and 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**

Beckman Coulter logo, COULTER, COULTER CLONE, CYTO-STAT, CYTO-TROL, EPICS, Flow-Count, IMMUNOPREP, IMMUNO-TROL, IOTest, MULTI-Q-PREP, Q-PREP, TQ-Prep, XL, and XL-MCL are trademarks of Beckman Coulter, Inc.

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