CYTO-STAT®/
COULTER CLONE®
CD8-ECD
IOTest® CD8-PC7

REF **737659 – 50 tests**
REF **737661 – 100 tests**

PN 737683-CA

	CD8-ECD	CD8-PC7
Specificity	CD8	CD8
Clone	SFCI21Thy2D3[5,6,22,23]	SFCI21Thy2D3[5,6,22,23]
Hybridoma	NS/1-AG4 x BALB/c	NS/1-AG4 x BALB/c
Immunogen	Human thymocytes	Human thymocytes
Ig Chain	IgG1[23]	IgG1[23]
Species	Mouse	Mouse
Source	Conditioned Media	Conditioned Media
Purification	Affinity chromatography	Affinity chromatography
Fluorescence	Excites at 486-580 nm / Emits at 610-635 nm	Excites at 486-580 nm / Emits at 710-800 nm
Conjugation	ECD (Phycoerythrin-Texas Red®-X)	PC7 (Phycoerythrin-Cy7)
Molar Ratio	ECD/Protein 0.5-1.5	PC7/Protein 0.5-1.5
Scatter Detection	Forward and/or side	Forward and/or side

MONOCLONAL ANTIBODY

NOT FOR DISTRIBUTION IN THE U.S.A. OR CANADA

For *In Vitro* Diagnostic Use

INTENDED USE

CYTO-STAT/COULTER CLONE CD8-ECD (CD8) or IOTest CD8-PC7[1] is a fluorescent murine monoclonal antibody reagent used to identify and enumerate the percentage of CD8+ (suppressor/cytotoxic) lymphocytes in whole blood by flow cytometry.

SUMMARY AND EXPLANATION

The lymphocyte population of human peripheral blood is composed of three cell types - T (thymus-derived), B (bone marrow-derived), and null cells. These cell types are morphologically indistinguishable by microscopy but can be identified by characteristic antigenic differences in their cell membranes.

Two main types of T lymphocytes can be distinguished according to their function and surface proteins. These are the inducer (CD4+) and suppressor/cytotoxic (CD8+) T lymphocytes.[2,3]

CD8

The CD8 antigen has a molecular weight of 76 kd.[1,4] It is normally present on a majority of thymocytes (approximately 80%)[5] and approximately 30-35% of peripheral blood T lymphocytes.[4,5] The CD8+ lymphocytes play a central role in regulating the immune response through suppressor and cytotoxic action.[2,3,6] The CD8 antigen reacts with the class I major histocompatibility complex (MHC) antigen on target cells.[4]

CLINICAL RELEVANCE

CD8

Identification of abnormal levels of CD8+ lymphocytes may aid in the diagnosis and/or prognosis of immunodeficiency diseases such as agammaglobulinemia, thymic aplasia (DiGeorge syndrome) and severe combined immunodeficiency.[7,8] The finding that increased levels of CD8+ cells are associated with viral infections such as hepatitis B, Epstein-Barr, and cytomegalovirus may also be of diagnostic and/or prognostic significance.[9,10]

CD4/CD8

Disease-related changes in CD4+ (CD4) and/or CD8+ (CD8) lymphocyte levels may alter CD4/CD8 inducer: suppressor/cytotoxic cell ratios. As a result, CD4/CD8 ratios may also be useful as diagnostic and/or prognostic indicators of immune competence.

CD4/CD8 ratios in conjunction with CD4+ lymphocyte cell numbers have been the most widely used laboratory parameters for the evaluation of AIDS-related complex and AIDS.[9,11] CD4/CD8 ratios fall toward zero in advanced AIDS patients with no detectable levels of CD4+ lymphocytes.[9] In such cases, CD8+ lymphocyte levels may be normal, increased, or decreased.

Modulations in CD4/CD8 ratios and CD4+ and CD8+ lymphocyte levels may occur in autoimmune diseases such as multiple sclerosis (MS) and systemic lupus erythematosus (SLE). Increased CD4/CD8 ratios and decreased numbers of CD4+ and CD8+ lymphocytes have been observed in patients with progressive (active) MS.[12-14] The lymphocyte response pattern in SLE, however, appears to reflect clinical disease activity and the level of organ involvement in the SLE disease process.[15-19] To illustrate, high CD4/CD8 ratios and elevated CD4+ lymphocyte percentages have been found in active/inactive SLE patients with multi-system disease including lymphadenopathy but little or no renal disease.[15,16] High CD4/CD8 ratios but decreased percentages of CD8+ lymphocytes have also been documented in similar active SLE patients.[17] Further, high CD4+ and low CD8+ lymphocyte percentages have been measured in active SLE patients with central nervous system disease but no renal disease.[18] In contrast, low CD4/CD8 ratios and decreased CD4+ lymphocyte percentages have been noted in active/inactive patients with SLE manifested by severe renal disease and/or thrombocytopenia.[15,16] In other active/inactive SLE patients, both low CD4+ and high CD8+ lymphocyte percentages have been recorded.[19] Finally, normal CD4/CD8 ratios have been obtained in patients with widespread multi-system SLE which often includes the renal and central nervous systems.[15,16]

High CD4/CD8 ratios have been found in patients with thymic aplasia.[7]

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]

Low CD4/CD8 ratios and decreased percentages of CD4+ lymphocytes have been documented in patients during phenotypic reconstitution following purged autologous 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% NaN_3, 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. Harmful if swallowed.
9. After contact with skin, wash immediately with plenty of water.
10. 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.
11. Use Good Laboratory Practice (GLP) when handling reagent.
12. 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/COULTER CLONE OR IOTEST MONOCLONAL ANTIBODY

MATERIAL SUPPLIED

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

MATERIALS REQUIRED BUT NOT SUPPLIED

Erythrocyte Lytic Reagent (as appropriate):

COULTER® IMMUNOPREP™ Reagent System for COULTER Q-PREP™ Workstation,
PN 7546946 - 100 tests

Diluent (if necessary) Autologous plasma
OR

COULTER IMMUNOPREP Reagent System for COULTER MULTI-Q-PREP™ or TQ-Prep™ Workstation,
PN 7546999 - 300 tests

Diluent (if necessary) Autologous plasma

Flow-Count™ Fluorospheres, PN 7547053
(Optional Reagent)

Isotypic Control MsIgG1-ECD
OR

IOTest IgG1-PC7 Isotypic Control,
PN 737662

COULTER CYTO-TROL™ Control Cells,
PN 6604248
OR

IMMUNO-TROL™ Control Cells, PN 6607077
OR

IMMUNO-TROL Low Control Cells, PN 6607098

Phosphate Buffered Saline (PBS), PN 6603369

12 x 75 mm test tubes

Blood collection tube with anticoagulant
(EDTA is recommended)

Transfer pipets

Vortex mixer

Centrifuge

Flow cytometer (See Instrument Requirements section)

Cell counter or hemocytometer

Filters for EPICS™ XL™/XL-MCL™ flow cytometer, 755 nm bandpass, PN 3814358, and 500 nm laser blocking filter, PN 3814357, to collect PC7 fluorescence

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 COULTER 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) - Buffy 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) Completely 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/COULTER CLONE 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 COULTER 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.

5. Lyse the red blood cells in each test tube using the procedure recommended for the lysing method selected (COULTER IMMUNOPREP Reagent System with the COULTER Q-PREP, MULTI-Q-PREP or TQ-Prep Workstation). The CD8-ECD sample is ready for flow cytometry analysis. Continue below for CD8-PC7 preparation.

Continued Preparation for CD8-PC7

6. Centrifuge samples at 400 x g for 4 minutes at room temperature.
7. Aspirate or decant supernatant and vortex.
8. Resuspend cell pellet by adding 1 mL of PBS.

Flow Cytometry Analysis

CAUTION: If the laser on the flow cytometer is misaligned or the gates improperly set, results may be erroneous.

Analyze cells on a flow cytometer properly standardized and gated on lymphocytes according to the instrument manual.

QUALITY CONTROL PROCEDURE

COULTER CYTO-TROL Control Cells (PN 6604248), IMMUNO-TROL Control Cells (PN 6607077), or IMMUNO-TROL Low Control Cells (PN 6607098) should be used as a positive control material to ensure proper working conditions. Alternatively, a normal, apparently healthy donor may be run as a positive control to ensure proper working conditions. Normal ranges should be established within a local population of normal donors.

Specific and/or nonspecific antibody Fc binding to monocytes and granulocytes in a sample can be excluded by proper gating on lymphocytes on the flow cytometer.

An appropriate CYTO-STAT/COULTER CLONE isotypic control is used to negate nonspecific antibody Fc binding to lymphocytes in each sample. The brightly fluorescent positively-stained lymphocyte population is measured in gates set to exclude the low level of nonspecific fluorescence.

Nonspecific fluorescence above the background (when cursor is set to gate out 98 ±1% nonspecific staining) is usually limited to 1-2% in normal individuals. Higher values may be seen in some neoplastic diseases. If the background level above the cursor for any control sample is greater than 1-2%, test results may be erroneous.

LIMITATIONS

1. For optimal results, blood samples should be stained within 6 hours of collection. Retain samples in blood collection tubes at room temperature prior to staining and analyzing. Do not refrigerate. Stored or refrigerated samples may give aberrant results. To ensure maximum viability, analyze stained cells promptly.
2. Certain patients may present special problems due to altered or very low numbers of certain cellular populations.
3. These reagents should not be diluted, aliquoted, or frozen. Use only as packaged.
4. These reagents are for flow cytometry use only.
5. These reagents are designed for use with whole blood preparations. They are not recommended for use with fresh or frozen mononuclear cell preparations.
6. Abnormal states of health are not always represented by abnormal percentages of certain leukocyte populations. An individual in an abnormal state of health may show the same leukocyte percentages as a healthy person. Use test results in conjunction with clinical and other diagnostic data.
7. All red blood cells may not lyse under the following conditions: nucleated red blood cells, abnormal protein concentration or hemoglobinopathies. This may cause

falsely 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			
	n	Min	Max	Mean ±1 SD
CYTO-STAT/COULTER CLONE CD8-ECD %CD8+ Lymphocytes	20	18	41	28.9 ±6.9
IOTest CD8-PC7 %CD8+ Lymphocytes	15	18	48	33.7 ±8.3

PERFORMANCE CHARACTERISTICS

SPECIFICITY

The SFCI21Thy2D3 (CD8) monoclonal binds to a nonpolymorphic domain of the MHC Class I molecules. SFCI21Thy2D3 (CD8) was assigned to CD8 during the 1st HLDA Workshop on Human Leucocyte Differentiation Antigens.[14]

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, 7 to 7,100 cells, and the percent CD8+ cells were measured over this range. The appropriate range of CD8-PC7 positive cells, 9 to 9,123 cells, and the percent CD8+ cells were measured over this range. Aliquots were stained and the linear regression between the expected values and the observed values 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	Linear regression	Linearity (R^2)	CD8 Cells (Range Tested x10^3)
CD8-ECD	Y=1.0016x + 22.5	0.9998	0.007 - 7.1
CD8-PC7	Y = 0.9965x + 1.12	0.9999	0.009 - 9.1

PRECISION

Within Run (Intralaboratory) CD8-ECD and CD8-PC7

On the same day and using the same flow cytometer, 10 measurements of the percentage of staining of a positive target (peripheral blood lymphocytes) were carried out. The results obtained are summarized in the following table:

Level	Number	Mean (%)	SD	CV (%)
CD8-ECD Lymphocytes	10	28	1.0	3.5
CD8-PC7 Lymphocytes	10	26	0.7	2.6

Interlaboratory CD8-ECD and CD8-PC7

On the same day and for the same positive target (peripheral blood lymphocytes), 10 measurements of the percentage of stained cells were carried out by two technicians and the preparations analyzed using two different cytometers. The results obtained are summarized in the following tables:

CYTO-STAT/COULTER CLONE CD8-ECD

Lab/Instrument	n	CD8+	Mean % ±1 SD	%CV
1 XL-MCL	10	28	1.0	3.5
2 XL-MCL	10	28	1.0	3.7

IOTest CD8-PC7

Lab/Instrument	n	CD8+	Mean % ±1 SD	%CV
1 XL-MCL	10	26	0.7	2.6
2 XL-MCL	10	27	0.5	1.9

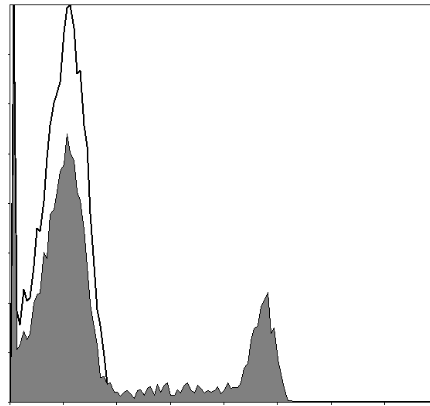
EXAMPLES

The graphs below are monoparametric representations (Count vs Fluorescence intensity) of a lysed normal whole blood sample.

Staining below is with IOTest CD8-PC7 Conjugated Antibody (PN 737661). Gate is on lymphocytes. A mouse PC7-conjugated IgG1 isotypic control (PN 737662) is shown.

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

LYMPHOCYTES

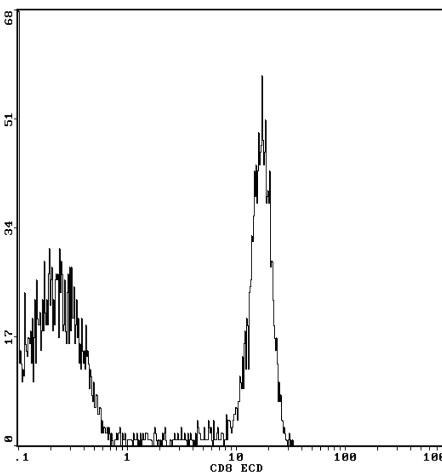


CD8-PC7

Staining below is with CYTO-STAT/COULTER CLONE CD8-ECD Conjugated Monoclonal Antibody (PN 737659). Gate is on lymphocytes. A mouse ECD-conjugated IgG1 isotypic control may be used but is not shown.

Acquisition and analysis for CD8-ECD are performed with a COULTER EPICS XL-MCL flow cytometer.

LYMPHOCYTES



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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

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