Solastra T Lineage Kit

REF A66287 – 25 tests

PN 775447-AB

	CD2-FITC	CD56-PE	CD7-ECD	CD5-PC5.5	CD45-PC7	CD8-FITC	CD4-PE	CD3-PC5.5
Specificity	CD2	CD56	CD7	CD5	CD45	CD8	CD4	CD3
Clone	39C1.5	N901/NKH-1	8H8.1	BL1a	J.33	SFCI21Thy2D3	SFCI12T4D11	UCHT1
Hybridoma	P3-x63-Ag.8.653 x rat spleen cells	NS-1 x BALB/c	P3 x 63.Ag8 x BALB/c	SP2/0-Ag14 x BALB/c	NS-1 x BALB/c	NS-1 x BALB/c	NS-1 x BALB/c	NS-1 x BALB/c
Immunogen	PHA-stimulated human lymphoblasts	Human chronic myeloid leukemia cells	Human thymocytes	Human thoracic duct lymphocytes	Laz 221 cell line	Human thymocytes	Human peripheral T lymphocytes	Human thymocytes and peripheral blood lymphocytes from a person with Sezary cell leukemia
Ig Chain	IgG2a	IgG1	IgG2a	IgG2a	IgG1	IgG1	IgG1	IgG1
Species	Rat	Mouse	Mouse	Mouse	Mouse	Mouse	Mouse	Mouse
Source	Ascites fluid	Ascites fluid	Ascites fluid	Ascites fluid	Ascites fluid	Conditioned media	Conditioned media	Conditioned media
Purification	Affinity chromatography	Affinity chromatography	Affinity chromatography	Affinity chromatography	Affinity chromatography	Affinity chromatography	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	Excites at 486-580 nm Emits at 610-635 nm	Excites at 486-580 nm Emits at 680-710 nm	Excites at 486-580 nm Emits at 750-790 nm	Excites at 468-509 nm Emits at 504-541 nm	Excites at 486-580 nm Emits at 568-590 nm	Excites at 486-580 nm Emits at 680-710 nm
Conjugation	FITC (Fluorescein Isothiocyanate)	PE (Phycoerythrin)	ECD (Phycoerythrin - Texas Red-X)	PC5.5 (Phycoerythrin-Cy5.5)	PC7 (Phycoerythrin-Cy7)	FITC (Fluorescein Isothiocyanate)	PE (Phycoerythrin)	PC5.5 (Phycoerythrin-Cy5.5)
Molar Ratio	FITC/Protein: 3-10	PE/Protein: 0.5-1.5	ECD/Protein: 0.5-1.5	PC5.5/Protein: 0.5-1.5	PC7/Protein: 0.5-1.5	FITC/Protein: 5-10	PE/Protein: 0.5-1.5	PC5.5/Protein: 0.5-1.5

MONOCLONAL ANTIBODY

For *In Vitro* Diagnostic Use

INTENDED USE

Solastra TL1 CD2-FITC/CD56-PE/CD7-ECD/CD5-PC5.5/CD45-PC7 and Solastra TL2 CD8-FITC/CD4-PE/CD3-PC5.5/CD45-PC7 Monoclonal Antibody Reagents are intended for use on the Cytomics FC 500 flow cytometry system. The reagent kit combines two reagent formulations (one four-color and one five-color) of fluorescent-labeled monoclonal antibodies. It is intended "For In Vitro Diagnostic Use" for the identification and enumeration of CD2+, CD3+, CD3+CD4+, CD5+, CD7+, CD3+CD8+ and CD56+ percentages on leukocytes. The kit is used as an aid in the differential diagnosis of patients with abnormal hematology indicative of anemia, leukopenia, thrombocytopenia, pancytopenia, neutrophilia, monocytosis, lymphocytosis, eosinophilia, thrombocytosis, lymphadenopathy and/or the presence of blasts. The Solastra immunophenotyping results are intended to be used in conjunction with other clinical findings and laboratory tests.[1,2]

SUMMARY AND EXPLANATION

Cell surface antigens appear to be acquired and lost by T lymphocytes in a manner reflecting the maturational (differentiation) and/or functional state of the cell. Once acquired, the same cell may coexpress some or all of these antigens for varying periods of time.

CLINICAL RELEVANCE

The T lineage markers in the Solastra kit are valuable flow cytometric immunophenotyping tools. In clinical indications in which a hematolymphoid neoplasia is known or suspected, these reagents, in conjunction with morphological classification, clinical history, and specimen type, allow effective evaluation with an appropriate degree of sensitivity. The Solastra T Lineage kit may be used in combination with the B Lineage (PN A66286) and/or Myelomonocytic Lineage (PN A66288) kits or alone depending on the clinical indications presented. The specific choices and combinations in the Solastra kits are based on the guiding principles of (1) addressing the clinical indications, (2) accounting for all major cell populations present in the specimen, and (3) providing sufficiently comprehensive identification of all major categories of hematopoietic cell populations in both normal and neoplastic states relevant to the clinical circumstances.[1,2]

The utilization of flow cytometric analyses have become an integral part of the standard of care in hematopathology. Because the clinical presentation of such neoplasms as chronic and acute leukemias, lymphomas, myelodysplasia syndrome, and myeloproliferative disease can vary so widely, a strategy of differential diagnosis, in which related neoplasms and non-neoplastic etiologies is generally considered. For this reason, such clinical indications as anemia, leukopenia, thrombocytopenia, pancytopenia, neutrophilia, monocytosis, lymphocytosis, eosinophilia, thrombocytosis, lymphadenopathy, and presence of blasts are investigated by flow cytometry in order to provide data critical to the diagnosis, staging, and prognosis of hematolymphoid neoplasias.[1,2]

PRINCIPLES OF TEST

This test depends on the ability of a monoclonal antibody to bind to the surface of cells expressing discrete antigenic determinants. Specimens are washed as appropriate to remove endogenous plasma proteins that may interfere with the specific binding. Specific cell staining is accomplished with the appropriate monoclonal antibody reagent. The Solastra T Lineage kit is composed of two combinations containing four or five monoclonal antibody reagents each conjugated to a specific fluorochrome and specific for different cell surface antigens.

Red blood cells are lysed, if necessary, with the VersaLyse Lysing Solution. The remaining white blood cells are analyzed by flow cytometry using an appropriate combination of subpopulation gates. In the first histogram, the population gates are identified based on a combination of CD45+ fluorescence intensity and Side Scatter (SS) characteristics.

Dual parameter histograms gated on the selected CD45/SS populations are used to determine the percentage of positively stained cells for each of the surface antigens recognized by the antibodies within the kit.

REAGENTS

See table above.

REAGENT CONTENTS

Contact Beckman Coulter Customer Service to obtain the antibody concentration.

The concentration of nonantibody reagents is 0.2% BSA, 0.01 M potassium phosphate, 0.15 M NaCl, 0.1% NaN_3 and stabilizers.

STATEMENT OF WARNINGS

1. These reagents contain 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 pipette 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 reagent.
8. Review all histograms before reporting results.
9. Harmful if swallowed.
10. After contact with skin wash immediately with plenty of water.

STORAGE CONDITIONS AND STABILITY

Unopened reagent is stable to the expiration date on the vial label 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. Do not freeze. Minimize exposure to light.

EVIDENCE OF DETERIORATION

Any change in the physical appearance of these reagents (normal appearance is a clear, pink liquid) or any major variation in values obtained for control samples may indicate deterioration and the reagent should not be used.

REAGENT PREPARATION

No preparation is necessary. The Solastra reagents are used directly from the vial. Bring reagent to 20-25°C prior to use.

SPECIMEN COLLECTION

- Each flow cytometric analysis requires 100 µL of whole blood, bone marrow or single lymphoid cell suspension.
- Avoid contamination of the tops and sides of the test tubes with blood, or incomplete lysis may occur.
- Staining may be performed on specimens with white blood cell counts in the range of 2-20 x 10^3 cells/µL.
- White blood cell counts exceeding 20 x 10^3 cells/µL require dilution.
- For optimum results, white blood cell counts below 2 x 10^3 cells/µL require centrifugation and resuspension prior to staining.
- Whole Blood and bone marrow may be collected using EDTA, Heparin or ACD anticoagulants as appropriate for the specimen.
- 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.

PROCEDURE FOR IMMUNOFLUORESCENCE CELL SURFACE STAINING WITH SOLASTRA MONOCLONAL ANTIBODY REAGENT

MATERIAL SUPPLIED

Solastra T Lineage Kit

A66287

Solastra TL1 CD2-FITC/CD56-PE/CD7-ECD/CD5-PC5.5/CD45-PC7 – 25 tests (0.5 mL)

Solastra TL2 CD8-FITC/CD4-PE/CD3-PC5.5/CD45-PC7 – 25 tests (0.5 mL)

MATERIALS REQUIRED BUT NOT SUPPLIED

VersaLyse Lysing Solution, PN A09777

IOTest 3 Fixative Solution, PN A07800

Solastra QuickCOMP 5 Kit, PN A83571

OR

QuickCOMP 4 Kit (except for CD45-PC5), PN 177017, with CD45-PC5.5, PN A62835, and CD45-PC7, PN IM3548

Blood collection tubes with anticoagulant (EDTA, ACD, or Heparin recommended)

Heat Inactivated Fetal Calf Serum

Phosphate Buffered Saline (PBS), PN 6603369

Heat Inactivated Mouse Serum

Transfer pipettes

Pasteur pipette

Micropipettors

12 x 75 mm test tubes

Conical centrifuge tube (15 mL)

Centrifuge

Vortex mixer

Flow cytometer

Cell counter or hemacytometer

Single Laser Filter Kit for Cytomics FC 500 flow cytometer only, PN 179044

OR

Single Laser Filter Block Assembly for Cytomics FC 500 flow cytometer only, PN 179045

Flow-Check Pro Fluorospheres, PN A63493

Flow-Set Pro Fluorospheres, PN A63492

Cotton tip applicators

PREPARATION OF REAGENTS

1. **PBS/2% Fetal Calf Serum (FCS) Wash Buffer:**
   a. Prepare a solution of PBS with 2% heat inactivated FCS for use as the wash buffer (1:50 v/v FCS/PBS).
2. **"Fix-and-Lyse Mixture":**
   a. Prepare the "Fix-and-Lyse" mixture (1 mL of mixture per tube) by adding 25 µL of UNDILUTED IOTest 3 10X Fixative Solution to 1 mL of VersaLyse Lysing Solution. Vortex the mixture for 3-5 seconds. Store the mixture at room temperature for up to 5 days after preparation.
3. **0.1% Formaldehyde PBS Resuspension Buffer:**
   a. Prepare a sufficient volume of 0.1% formaldehyde PBS resuspension buffer by diluting 12.5 µL of the IOTest 3 Fixative Solution at its 10X concentration in 1 mL of PBS.

PROCEDURE

SPECIMEN PREPARATION

Both whole blood and bone marrow specimens are pre-washed prior to staining to avoid plasma/serum protein interferences. Based on the individual laboratory workflow, specimens can be washed using a bulk or single tube procedure.

NOTE: Single cell suspensions prepared from lymphoid tissues may not require washing prior to staining if the specimen was washed during the disaggregation process. If washing steps were not performed for removal of residual soluble proteins, or if the cells were resuspended into a buffer containing human serum or serum proteins, then pre-washing is necessary. Follow your laboratory procedure for washing.

NOTE: To minimize non-specific Fc binding, a solution of PBS/50% mouse serum (50:50 v/v heat inactivated mouse serum/PBS) may be used as the resuspension buffer.

CAUTION: Failure to follow the washing instructions (volumes and wash cycles) may cause erroneous results.

Bulk Wash Procedure

1. Obtain WBC count of the sample.
2. Add 1.0 mL whole blood or bone marrow specimen to a 15 mL conical centrifuge tube.
3. Add 9.0 mL of the PBS/2% FCS wash buffer. Mix by gentle inversion.
4. Centrifuge at 150 x g for 10 minutes.
5. Aspirate and discard supernatant.
6. Repeat steps 3-5 two additional times.
7. Resuspend the washed pellet in either PBS/2%FCS or PBS/50% mouse serum with an appropriate volume to obtain a WBC count between 2-20 x 10^3 cells/µL.
8. Proceed to Staining Procedure.

Single Tube Wash Procedure

1. Obtain WBC count of the sample.
   a. If the WBC count is above 20 x 10^3 cells/µL, dilute sample appropriately with the PBS/2% FCS wash buffer.
   b. If the WBC count is <2 x 10^3 cells/µL, the sample must be concentrated prior to washing.
2. For each sample add 100 µL of whole blood or bone marrow specimen to two 12 x 75 mm test tubes labeled for each of the Solastra T Lineage Reagents (TL1 and TL2).
3. Add 3.0 mL of the PBS/2% FCS wash buffer. Mix by gentle inversion.
4. Centrifuge at 1000 x g for 2 minutes.
5. Aspirate and discard supernatant.
6. Repeat steps 3-5 two additional times.
7. Resuspend the washed pellet in either PBS/2%FCS or PBS/50% mouse serum to the initial 100 µL volume.
8. Proceed to Staining Procedure.

STAINING PROCEDURE

1. For each sample washed using the Bulk Wash Procedure, and for the single cell suspensions of lymphoid tissues, label individual 12 x 75 mm test tubes for TL1 and TL2. For samples washed using the Single Tube Wash Procedure, proceed to step 3.
2. Add 100 µL of the sample to each test tube.

   IMPORTANT: If blood droplets remain around the top of the test tube they must be removed or unlysed red blood cells may contaminate the final sample and skew the results. A cotton tip applicator may be used for removal.

3. Add 20 µL of Solastra TL1 or Solastra TL2 to the corresponding labeled test tube.
4. Vortex gently. Incubate the reaction mixtures at 20-25°C for 15-20 minutes. Protect from light.
5. Lyse the red blood cells in each test tube.

   NOTE: Single cell suspensions from lymphoid tissues do not require the red blood cell lysis. Proceed to step e.

   a. Add 1 mL of the "Fix-and-Lyse" mixture to each test tube and vortex immediately for 1 second.
   b. Incubate at least 10 minutes at room temperature (20 – 25°C), protected from light.
   c. Centrifuge for 5 minutes at 150 x g at room temperature.
   d. Remove the supernatant by aspiration.
   e. Resuspend the cell pellet in 3 mL of PBS.
   f. Centrifuge for 5 minutes at 150 x g at room temperature.
   g. Remove the supernatant by aspiration and resuspend the cell pellet in 0.5 mL of 0.1% formaldehyde PBS buffer.
   h. To minimize the possibility of less than optimal results, analyze stained cells promptly.

6. Analyze cells on a flow cytometer properly standardized and gated on each population of interest. A recommended SAMPLE ANALYSIS PROCEDURE is given below.
   a. Fluorescence flow cytometry readings should be collected on a log scale.
   b. Side Scatter (SS) should be collected on a linear scale.
   c. Forward Scatter (FS) should be collected on a linear scale.

QUALITY CONTROL

1. Ensure the flow cytometer is properly aligned and standardized for light scatter and fluorescence intensities according to the manufacturer's recommendations.
2. Run Flow-Check Pro Fluorospheres to verify instrument alignment according to the package insert.
3. Set PMT voltages using Flow-Set Pro Fluorospheres according to the package insert.
4. Set compensation using appropriate reagents and methods.

a. Initial compensation levels can be established with a pre-washed normal blood donor sample prepared with the Solastra QuickCOMP 5 Kit using full matrix compensation.
b. Compensation may also be performed by staining separate aliquots of the normal blood sample with 20 µL each of the CD45-FITC, CD45-PE, and CD45-ECD from the QuickCOMP 4 reagents, and 10 µL each of CD45-PC5.5 and CD45-PC7.

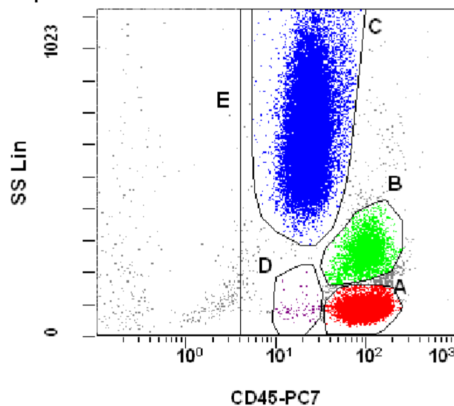
5. Before patient samples are analyzed, a pre-washed normal blood specimen stained with the Solastra T Lineage Kit reagents should be used to verify compensation and antibody reactivity. Manual adjustment to initial compensation values should be made during verification and used for the patient specimen testing.
6. Specific and/or nonspecific antibody Fc binding to monocytes and granulocytes in a sample can be minimized by resuspension of the pre-washed specimens in 50% mouse serum prior to staining. Refer to Specimen Preparation section.
7. Negatively stained populations may be used for proper cursor placement.[15]

FLOW CYTOMETRY ACQUISITION/ANALYSIS PROCEDURE

CAUTION: Erroneous results may occur if the laser on the flow cytometer is misaligned, inappropriate filters are present, or the gates are improperly set.

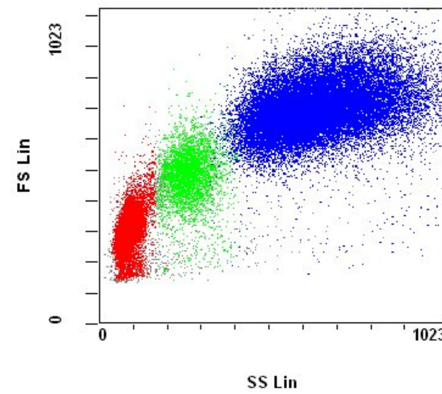
1. Create appropriate analysis protocols to define the population gates and the series of dual parameter histograms for analysis of the reagent specificities.
2. Collect a minimum of 50,000 CD45+ events. Stop count region may be set from the CD45-PC7 vs. SS dual parameter histogram (Gate E) (see Figure 1).
3. Collect CD45-PC7 vs. SS dual parameter histogram. Three or more discrete populations may be apparent in whole blood and bone marrow specimens; a single population is usually apparent in cell suspensions from lymphoid tissues. Draw gates around the leukocyte populations of interest based on the CD45+ fluorescence and SS characteristics (see Figure 1).

Figure 1: A dual parameter CD45-PC7 vs. SS histogram of a normal peripheral blood sample to identify lymphocytes (LY) (Gate A), monocytes (MO) (Gate B), granulocytes (GR) (Gate C), and CD45^{dim}/SS^{low} cells (Gate D) and region (Gate E) to define the stop count on CD45+ events.



4. A SS vs. FS histogram may also be collected to assess the prepared sample scatter characteristics (see Figure 2).

Figure 2: SS vs. FS histogram (Ungated)



5. It is important to assess CD45 dim to negative events to ensure they are not relevant populations. Comparison of populations in a SS vs. FS histogram versus CD45-PC7 vs. SS dual parameter histogram will aid in detecting populations that may be CD45 negative, but have the light scatter of a cell (see Figures 3a-3b); these populations should be included in Gate D on the CD45-PC7 vs. SS histogram.

Figures 3a–3b: Representative examples of an acute lymphocytic leukemia (ALL) peripheral blood sample. Gate D displays a large CD45 dim to negative population in the dual parameter CD45-PC7 vs. SS histogram. These events are above the threshold for lymphocytes in the SS vs. FS histogram.

Figure 3a: CD45-PC7 vs. SS Histogram (Ungated)

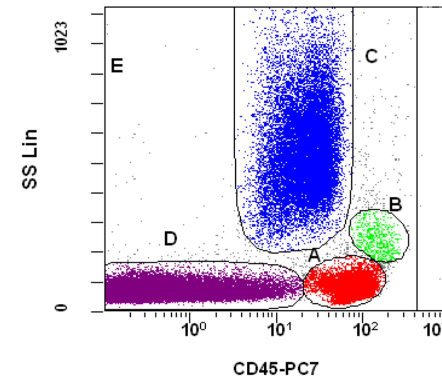
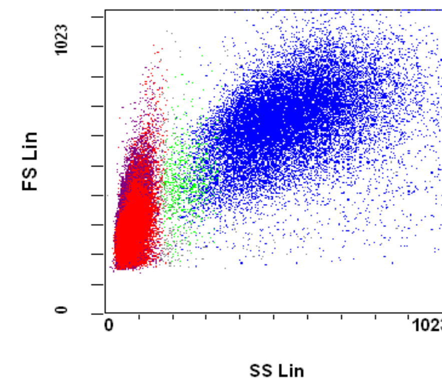


Figure 3b: SS vs. FS Histogram (Ungated)



6. When analyzing populations using gates other than quadstats (for example, manually created analysis regions), it is essential to ensure that the edge of the regions include the first channel of the axis of interest. In many cases significant populations of cells may be accumulated in the first channel.
7. For Solastra TL1 CD2-FITC/CD56-PE/CD7-ECD/CD5-PC5.5/CD45-PC7:
   a. Create dual parameter histograms gated on the appropriate populations created in Figure 1 to enumerate CD2+, CD56+, CD7+ and CD5+ (see Figures 4a-4f).
   b. The table below indicates the quadrants and histograms used to calculate each population.

Population	Quadrants	Histogram Figure #	
		Gated on LY	Gated on CD45^{dim}/SS^{low}
CD2+	2 + 4	4b	4e
CD56+	2 + 4	4c	4f
CD7+	1 + 2	4a	4d
CD5+	1 + 2	4b	4e

8. For Solastra TL2 CD8-FITC/CD4-PE/CD3-PC5.5/CD45-PC7:
   a. Create dual parameter histograms gated on the appropriate populations created in Figure 1 to enumerate CD3+CD8+, CD3+CD4+ and CD3+ (see Figures 5a-5b).
   b. The table below indicates the quadrants and histograms used to calculate each population.

Population	Quadrant(s)	Histogram Figure #
		Gated on LY
CD3+CD8+	2	5a
CD3+CD4+	2	5b
CD3+	2 + 4	5a

9. Use the negatively stained populations for placement of the quadstat or region positive boundaries (see Figures 4-5).

   NOTE: If all cells are positive for the specific marker, set the boundary based on a negatively stained population from a different marker conjugated to the same fluorochrome within the same lineage.

Figures 4a-4f: Dual parameter histograms from a normal peripheral blood sample for CD2-FITC vs. CD7-ECD, CD2-FITC vs. CD5-PC5.5 and CD56-PE vs. CD5-PC5.5.

Figure 4a: Tube TL1 gated on Lymphocytes (Gate A)

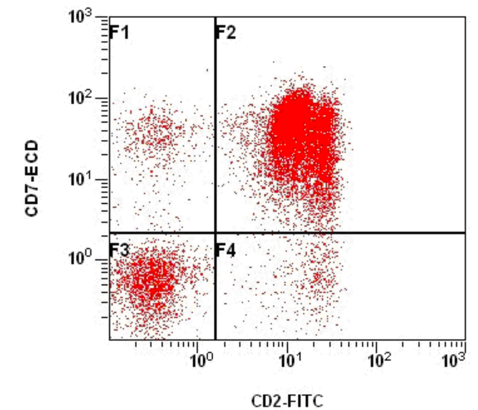


Figure 4b: Tube TL1 gated on lymphocytes (Gate A)

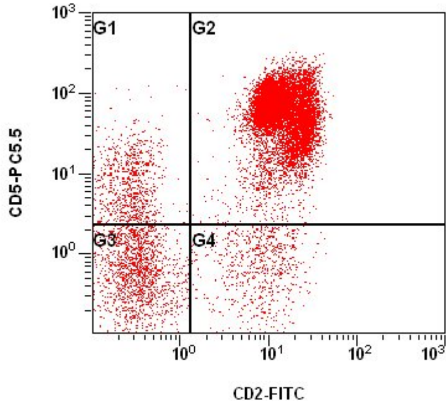


Figure 4c: Tube TL1 gated on lymphocytes (Gate A)

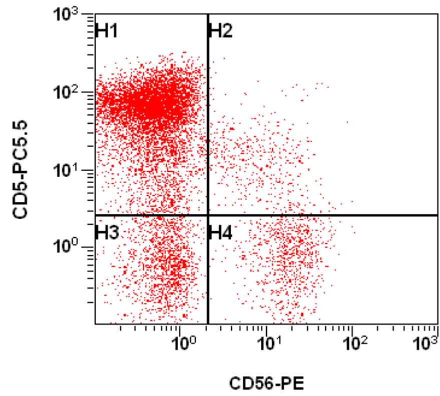


Figure 4d: Tube TL1 gated on $CD45^{dim}/SS^{low}$ (Gate D)

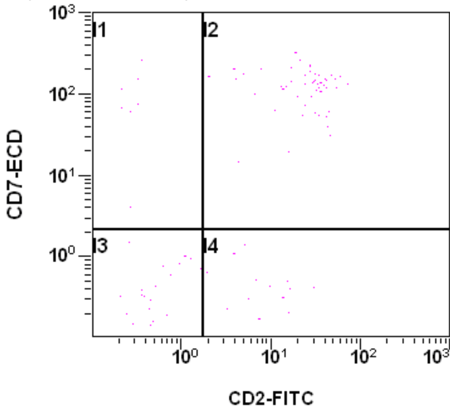


Figure 4e: Tube TL1 gated on $CD45^{dim}/SS^{low}$ (Gate D)

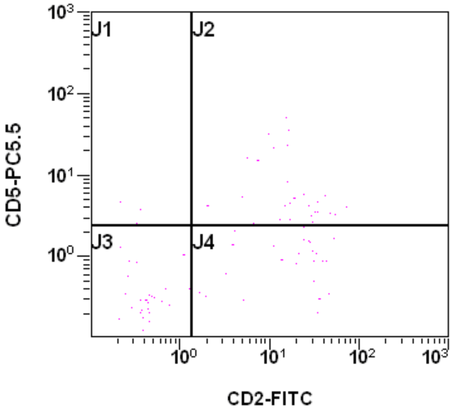
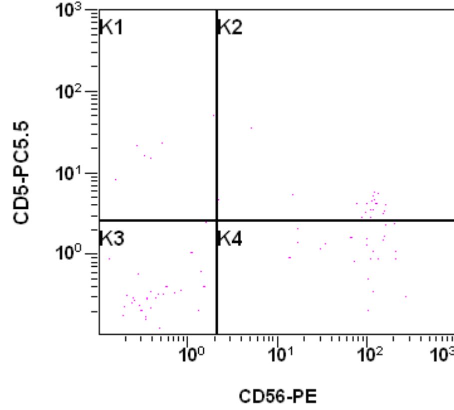


Figure 4f: Tube TL1 gated on $CD45^{dim}/SS^{low}$ (Gate D)



Figures 5a-5b: Dual parameter histograms from a normal peripheral blood sample for CD3-PC5.5 vs. CD8-FITC and CD3-PC5.5 vs. CD4-PE.

Figure 5a: Tube TL2 gated on lymphocytes (Gate A)

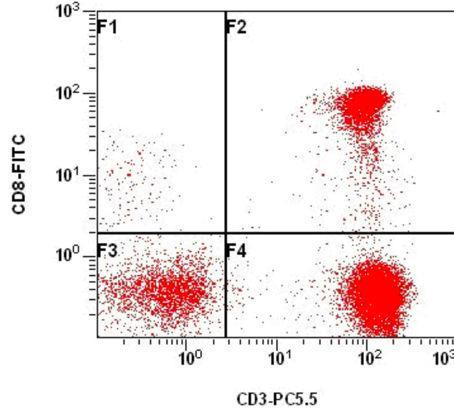


Figure 5b: Tube TL2 gated on lymphocytes (Gate A)

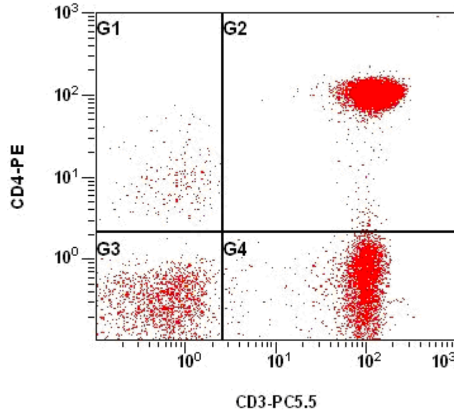
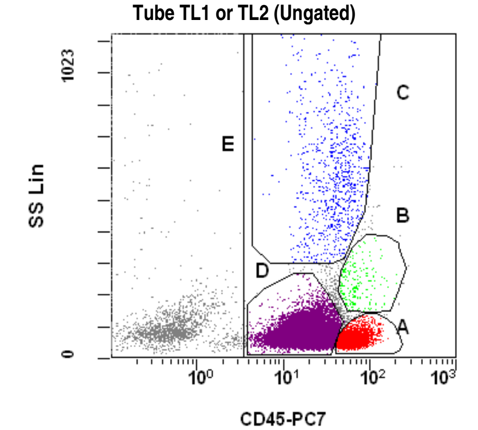


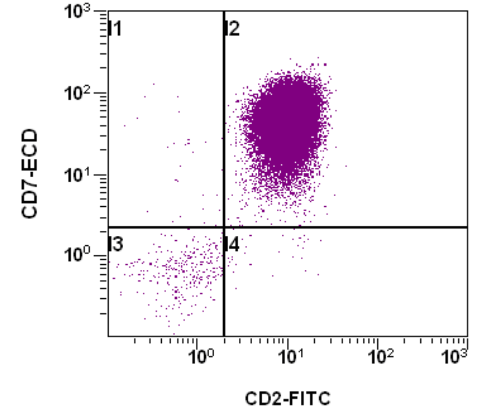
Figure 6: Representative examples of dual parameter histograms from a precursor T-cell lymphoblastic leukemia / lymphoma (LL / LBL) bone marrow sample for Solastra TL1 and TL2 reagents.

NOTE: Not all dual parameter histograms shown.

Tube TL1 or TL2 (Ungated)



Tube TL1 gated on $CD45^{dim}/SS^{low}$ (Gate D)



Tube TL1 gated on $CD45^{dim}/SS^{low}$ (Gate D)

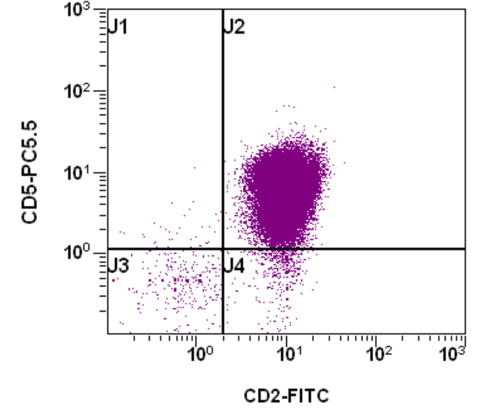
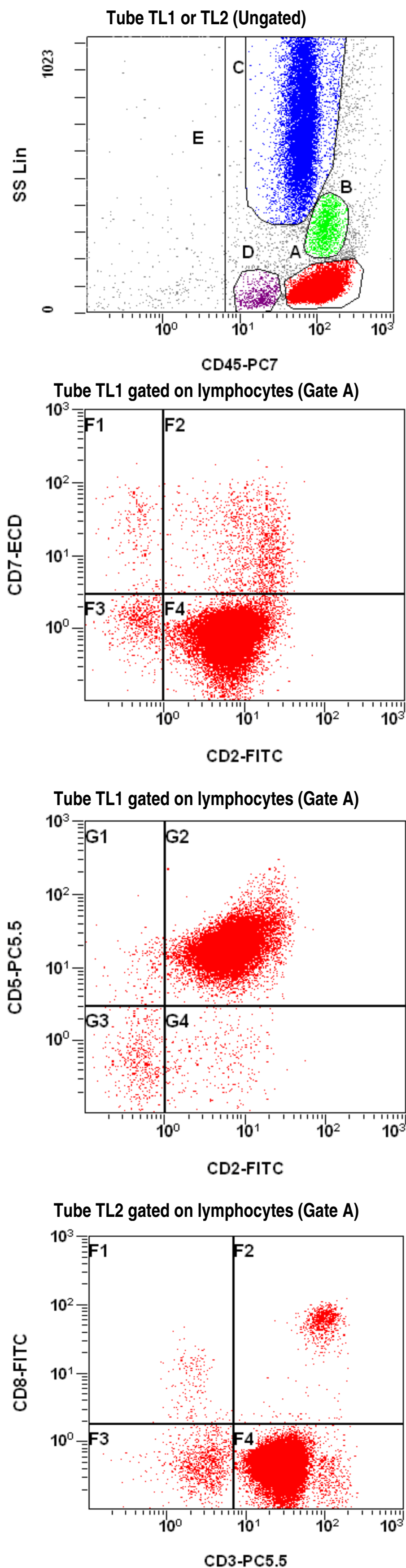
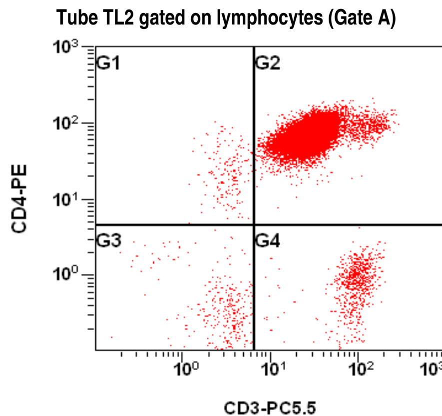


Figure 7: Representative examples of dual parameter histograms from a mature T-cell neoplasm peripheral blood sample for Solastra TL1 and TL2 reagents.

NOTE: Not all dual parameter histograms shown.





LIMITATIONS

1. EDTA collected specimens stained with Solastra TL1 or Solastra TL2 may be prepared within 24 hours. Analysis of samples must be performed on the same day as sample staining.
2. Specimens collected with Heparin or ACD may be prepared within 48 hours of collection. Analysis of samples must be performed on the same day as sample staining.
3. Use of Solastra reagents on the FC 500 flow cytometer requires Single Laser Filter Kit or Single Laser Filter Block Assembly.
4. Retain specimens at room temperature prior to sample preparation.
5. Do not refrigerate specimens. Refrigerated specimens may give aberrant results.
6. Minimize the possibility of less than optimal results by analyzing stained cells promptly.
7. Recommended cell viability for venous blood specimens is >90%, but this may be difficult to achieve with certain abnormal specimens.
8. Solastra monoclonal antibody reagents are designed for use with whole blood, bone marrow and single cell suspensions of lymphoid populations.
9. Do not dilute, aliquot, or freeze the reagents. Use only as packaged.
10. Prolonged exposure of cells to lytic reagents may cause white blood cell destruction and loss of cells in the population of interest.
11. 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.
12. In patients treated with anti-human monoclonal antibody therapies, detection of the specific targeted antigens may be diminished or absent due to partial or complete blocking by the treatment antibody.[10,16,27]

EXPECTED VALUES

Whole blood specimens were collected from apparently healthy males and females with normal hematology laboratory results. The population was geographically diverse and included individuals who were unselected as to race or age.

Samples were stained with the Solastra T Lineage Kit reagents and analyzed by flow cytometry. Values are presented in the Normal Whole Blood Table below.

Expected values were not established for normal bone marrow or lymphoid tissues.

These are intended as representative values only. Each laboratory should establish its own expected values from the local population of normal donors.

Normal Whole Blood

Measurement	n	Mean	Interval	
			Lower	Upper
% Lymphocytes				
CD2+	130	85.24	72.63	95.35
CD5+	130	78.65	61.42	88.13
CD7+	130	81.53	69.74	91.04
CD56+	128	14.38	3.97	32.34
CD3+	130	77.82	62.42	88.43
CD3+CD4+	130	49.52	17.61	70.19
CD3+CD8+	130	26.68	12.01	52.40

PERFORMANCE CHARACTERISTICS

The performance characteristics data presented below was collected on Cytomics FC 500 Flow Cytometry System with CXP software.

SPECIFICITY

The CD45 antigen is expressed on every type of hematopoietic cell except mature erythrocytes and their immediate progenitors. It has not been detected in differentiated nonhematopoietic tissue.[3-6]

The CD2 antigen is an early, lineage-specific pan T cell surface antigen and normally is present on some bone marrow prothymocytes, on over 95% of thymocytes, on all peripheral T lymphocytes and on a subpopulation of natural killer cells.[7,17] Once present, the CD2 antigen is expressed continuously throughout T lymphocytes differentiation.[17] The CD2 antigen is not detected on peripheral blood B lymphocytes, monocytes, granulocytes or platelets.

The CD3 antigen is normally present on the cell surface of mature thymocytes and resting and activated peripheral blood mature T lymphocytes (both inducer and suppressor/cytotoxic populations).[18-20]

The CD4 antigen is present on thymocytes and the inducer T lymphocyte population in peripheral blood.[20,21] It is also expressed at low density on monocytes.[21]

The CD5 antigen is present on all mature T lymphocytes and on most thymocytes. CD5 is also present on a B lymphocyte subset but is not found on granulocytes or monocytes.[18-20]

CD7 antigen is found on thymocytes and on the majority of peripheral blood T lymphocytes. It is also expressed on most NK cells, a subpopulation of pre-B lymphocytes, on B-lymphocytes originating from fetal bone marrow, and on pluripotent hematopoietic stem cells.[22] Mature B lymphocytes, cells of erythrocytic, myeloid, and megakaryocytic lineage do not normally express CD7.[22]

The CD8 antigen is normally present on approximately 80% of thymocytes and approximately 30-35% of peripheral blood T lymphocytes and some NK cells.[23-25]

The CD56 antigen is expressed exclusively on a subpopulation of lymphocytes that demonstrate NK activity.[9-11] Virtually all of these cells capable of mediating non-TCR mediated cytotoxicity in peripheral blood express CD56.[12-14] This subpopulation consists of both NK cells (CD3-CD56+) and a small subset of T cells (CD3+CD56+).[9,11,14] CD56 is not expressed on other T or B lymphocyte, monocyte, granulocyte or erythrocyte populations.[14,25,26]

The antigen specificity of the CD45, CD2, CD56, CD7, and CD5 monoclonal antibodies comprising the Solastra TL1 reagent and the CD45, CD3, CD4, and CD8 monoclonal antibodies comprising Solastra TL2 reagent has been previously established by the Human Leukocyte Differentiation Antigen Workshops.[7]

To assess cellular cross-reactivity, the CD2, CD3, CD4, CD5, CD7, CD8, and CD56 monoclonal antibodies comprising Solastra TL1 or Solastra TL2 monoclonal antibody reagents were screened on normal human adult donor blood samples. Results consistently demonstrated that the CD2, CD3, CD4, CD5, CD7, CD8, and CD56 monoclonal antibodies reacted specifically with the appropriate lymphocyte populations. Monocytes were dimly stained with CD4 monoclonal antibody.

LINEARITY

Determination of linearity was performed in accordance with CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach. A sample containing positive cells (positive cell lines) and a negative cell target (fixed red blood cell pool) at a fixed cell count were mixed in different proportions to achieve a positive/negative cell ratio from 0 to 100%. Three replicate measurements were made at each of 10 equally spaced ratios for lower range (0 to 10%) and higher range (10 to 100%). Cells were stained with the Solastra T Lineage kit reagents and analyzed by flow cytometry (Cytomics FC 500). Percentages for each CD were measured for each cell mixture for assessment of linearity by regression analysis, results shown in the table below.

Specificity	Linear Regression	Linearity (R^2)
CD2	y=0.9826x – 0.0072	0.9974
CD5	y=0.9766x – 0.0101	0.9967
CD7	y=0.9803x – 0.0100	0.9967
CD56	y=0.9794x – 0.0053	0.9974
CD3	y=0.9785x + 0.0089	0.9986
CD4	y=0.9791x + 0.0089	0.9986
CD8	y=0.9778x + 0.0089	0.9986

LOWER LIMIT OF DETECTION

A study was conducted in accordance with CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. Results support a lower limit of detection of 0.3% when collecting 50,000 events.

ACCURACY OF METHOD

The degree of agreement between the Solastra T Lineage Reagent Kit and comparator reagents was studied in normal and abnormal blood, bone marrow and single cell suspensions of lymphoid tissues gated on the leukocyte populations defined by SS vs. CD45-PC7. For gated populations and markers yielding results ≥2%, the data provided in the tables below support the premise that the reagents are equivalent in their performance for enumerating CD2+, CD3+, CD3+CD4+, CD5+, CD3+CD8+ and CD56+ percentages on leukocytes.

ACCURACY OF METHOD

Measurement	n	Mean ± SD	Min	Max
% CD2+ gated on Lymphocytes (A)				
Solastra CD2+ (TL1)	215	67.81 ±26.72	2.38	99.21
CYTO-STAT CD2/CD20	215	67.78 ±26.84	3.98	99.28
% CD2+ gated on CD45^{dim}/SS^{low} (D)				
Solastra CD2+ (TL1)	18	13.13 ±20.43	2.76	92.19
CYTO-STAT CD2/CD20	18	13.08 ±20.03	2.66	90.68
% CD5+ gated on Lymphocytes (A)				
Solastra CD5+ (TL1)	221	68.46 ±20.86	3.74	99.82
COULTER CLONE CD5 (T1)-RD1	221	68.61 ±20.90	3.41	99.77
% CD5+ gated on CD45^{dim}/SS^{low} (D)				
Solastra CD5+ (TL1)	12	23.18 ±29.64	2.32	99.53
COULTER CLONE CD5 (T1)-RD1	12	22.85 ±29.96	2.09	99.62
% CD56+ gated on Lymphocytes (A)				
Solastra CD56+ (TL1)	174	15.44 ±9.65	2.05	53.46
tetraCHROME CD45/CD56/CD19/ CD3	174	15.82 ±9.94	2.41	56.60
% CD56+ gated on CD45^{dim}/SS^{low} (D)				
Solastra CD56+ (TL1)	17	41.84 ±40.31	3.05	94.45
tetraCHROME CD45/CD56/CD19/ CD3	17	42.09 ±41.33	2.47	95.92
% CD3+ gated on Lymphocytes (A)				
Solastra CD3+ (TL2)	218	61.41 ±24.26	2.73	95.72
tetraCHROME CD45/CD4/CD8/ CD3	218	62.12 ±24.36	2.56	96.19
% CD3+CD4+ gated on Lymphocytes (A)				
Solastra CD3+CD4+ (TL2)	216	36.29 ±17.03	2.12	92.09
tetraCHROME CD45/CD4/CD8/ CD3	216	36.49 ±17.00	2.16	94.89
% CD3+CD8+ gated on Lymphocytes (A)				
Solastra CD3+CD8+ (TL2)	215	23.95 ±14.53	2.05	67.74
tetraCHROME CD45/CD4/CD8/ CD3	215	24.25 ±14.53	2.00	70.12

Along with the accuracy of method study, an analysis of subset populations was performed on the same data set resulting in the performance characteristics in the table below.

ANALYSIS OF SUBSET POPULATIONS

Measurement	n	Mean ± SD	Min	Max
% CD7+ gated on Lymphocytes (A)				
Solastra CD7+ (TL1) Subset of CD2+	214	63.67 ±25.76	2.26	96.01
CYTO-STAT CD2/CD20	214	68.08 ±26.54	4.76	99.28
% CD7+ gated on CD45^{dim}/SS^{low} (D)				
Solastra CD7+ (TL1) Subset of CD45^{dim}/SS^{low}	6	41.60 ±32.43	2.85	90.37
tetraCHROME CD45/CD56/CD19/ CD3	6	67.55 ±21.32	43.44	95.61

PRECISION

Within Run Variability

The percent positive values were determined using IMMUNO-TROL, run in duplicate, twice each day for up to 20 days at 4 geographically diverse sites using the Solastra T Lineage Kit reagents.

Measurement	Repeatability (CV %)	Mean
% Lymphocytes		
CD2+	0.67%	81.78
CD5+	0.93%	72.73
CD7+	0.73%	74.65
CD56+	3.32%	13.76
CD3+	0.76%	71.16
CD3+CD4+	1.24%	45.48
CD3+CD8+	1.94%	22.67

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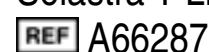
PRODUCT AVAILABILITY

Solastra T Lineage Kit
 A66287

Solastra TL1 CD2-FITC/CD56-PE/CD7-ECD/CD5-PC5.5/ CD45-PC7 – 25 tests (0.5 mL)

Solastra TL2 CD8-FITC/CD4-PE/CD3-PC5.5/ CD45-PC7 – 25 tests (0.5 mL)

Cy5.5 and Cy7 are licensed from GE Healthcare under patents 5,268,486, 5,569,587, and 5,627,027.

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