Solastra Myelomonocytic Lineage Kit

REF A66288 – 25 tests

PN 775448-AC

	CD15-FITC	CD11b-PE	CD16-ECD	CD14-PC5.5	CD45-PC7	HLA-DR-FITC
Specificity	CD15	CD11b	CD16	CD14	CD45	HLA-DR
Clone	80H5	Bear1	3G8	RMO52	J.33	Immu357
Hybridoma	MOPC.315-43 X BALB/c	SP2/O-Ag14 x BALB/c	SP2/O x BALB/c	SP2/O x BALB/c	NS-1 x BALB/c	P3-X63-Ag.8.653 x BALB/c
Immunogen	Human granulocytes	Purified human monocytes	Human neutrophils	Isolated human monocytes	Laz 221 cell line	Cell line transformed by EBV
Ig Chain	IgM	IgG1	IgG1	IgG2a	IgG1	IgG1
Species	Mouse	Mouse	Mouse	Mouse	Mouse	Mouse
Source	Ascites fluid	Ascites fluid	Ascites fluid	Ascites fluid	Ascites fluid	Ascites fluid
Purification	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
Conjugation	FITC (Fluorescein Isothiocyanate)	PE (Phycoerythrin)	ECD (Phycoerythrin - Texas Red-X)	PC5.5 (Phycoerythrin-Cy5.5)	PC7 (Phycoerythrin-Cy7)	FITC (Fluorescein Isothiocyanate)
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: 3-10

	CD56-PE	CD34-ECD	CD117-PC5.5	CD7-FITC	CD13-PE	CD33-PC5.5
Specificity	CD56	CD34	CD117	CD7	CD13	CD33
Clone	N901/NKH-1	581	104D2D1	8H8.1	366	D3HL60.251
Hybridoma	NS-1 x BALB/c	NSO x BALB/c	SP2/O x BALB/c	P3 x 63.Ag8 x BALB/c	NS-1 x BALB/c	NS-1 x BALB/c
Immunogen	Human chronic myeloid leukemia cells	KG-1a cell line + human CD34+ leukemia	MOLM-1 leukemic cell line	Human thymocytes	Human acute myelomonocytic leukemia cells	HL60 cell line
Ig Chain	IgG1	IgG1	IgG1	IgG2a	IgG1	IgG1
Species	Mouse	Mouse	Mouse	Mouse	Mouse	Mouse
Source	Ascites fluid	Ascites fluid	Ascites fluid	Ascites fluid	Ascites fluid	Ascites fluid
Purification	Affinity chromatography	Affinity chromatography	Affinity chromatography	Affinity chromatography	Affinity chromatography	Affinity chromatography
Fluorescence	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 468-509 nm Emits at 504-541 nm	Excites at 486-580 nm Emits at 568-590 nm	Excites at 468-509 nm Emits at 610-635 nm
Conjugation	PE (Phycoerythrin)	ECD (Phycoerythrin - Texas Red-X)	PC5.5 (Phycoerythrin-Cy5.5)	FITC (Fluorescein Isothiocyanate)	PE (Phycoerythrin)	PC5.5 (Phycoerythrin-Cy5.5)
Molar Ratio	PE/Protein: 0.5-1.5	ECD/Protein: 0.5-1.5	PC5.5/Protein: 0.5-1.5	FITC/Protein: 3-10	PE/Protein: 0.5-1.5	PC5.5/Protein: 0.5-1.5

MONOCLONAL ANTIBODY

For *In Vitro* Diagnostic Use

INTENDED USE

Solastra ML1 CD15-FITC/CD11b-PE/CD16-ECD/ CD14-PC5.5/CD45-PC7, Solastra ML2 HLA-DR-FITC/ CD56-PE/CD34-ECD/CD117-PC5.5/CD45-PC7, and Solastra ML3 CD7-FITC/CD13-PE/CD34-ECD/ CD33-PC5.5/CD45-PC7 Monoclonal Antibody Reagents are intended for use on the Cytomics FC 500 flow cytometry system. The reagent kit combines three five-color reagent formulations of fluorescent-labeled monoclonal antibodies. It is intended "For In Vitro Diagnostic Use" for the identification and enumeration of CD15+, CD11b+, CD16+, CD14+, CD34+, CD56+, HLA-DR+, CD117+, CD13+, CD7+ and CD33+ 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 myeloid lineage cells in a manner reflecting the maturational (differentiation) and/or functional state of the cell. Once acquired, the same cell may co-express some or all of these antigens for varying periods of time.

CLINICAL RELEVANCE

The myelomonocytic 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 Myelomonocytic Lineage kit may be used in combination with the B Lineage (PN A66286) and/or T Lineage (PN A66287) 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 Myelomonocytic Lineage kit is composed of three combinations each containing 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 on page 1.

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 Myelomonocytic Lineage Kit

REF A66288

Solastra ML1 CD15-FITC/CD11b-PE/CD16-ECD/CD14-PC5.5/CD45-PC7 - 25 tests (0.5 mL)

Solastra ML2 HLA-DR-FITC/CD56-PE/CD34-ECD/CD117-PC5.5/CD45-PC7 - 25 tests (0.5 mL)

Solastra ML3 CD7-FITC/CD13-PE/CD34-ECD/CD33-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 three 12 x 75 mm test tubes labeled for each of the Solastra Myelomonocytic Lineage Reagents (ML1, ML2 and ML3).
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 ML1, ML2 and ML3. 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 ML1, Solastra ML2 or Solastra ML3 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 Myelomonocytic 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.

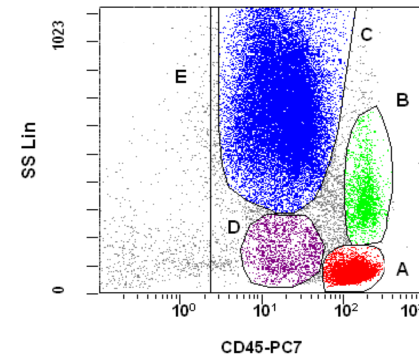
7. Negatively stained populations may be used for proper cursor placement.[16]

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.

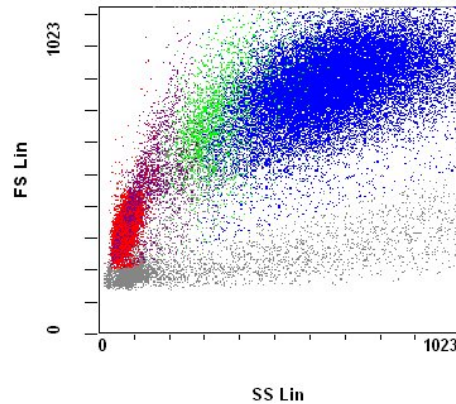
1. Create appropriate analysis protocols to define the population gates and the series of dual parameter histograms for analysis of the reagent specificities.
2. Collect a minimum of 50,000 CD45+ events. Stop count region may be set from the CD45-PC7 vs. SS dual parameter histogram (Gate E) (see Figure 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 an apparently normal bone marrow 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)

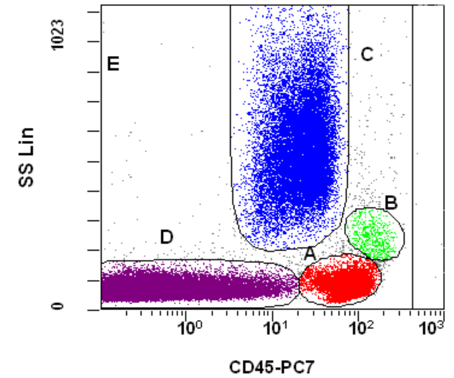
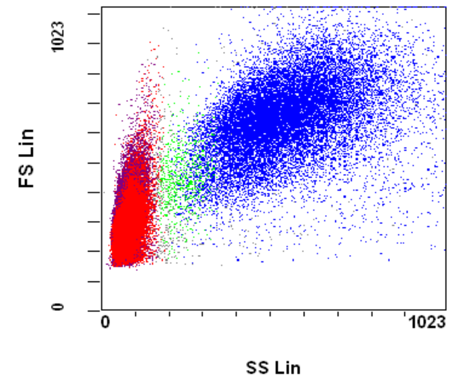


Figure 3b: SS vs. FS Histogram (Ungated)



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 ML1 CD15-FITC/CD11b-PE/CD16-ECD/CD14-PC5.5/CD45-PC7:
   a. Create dual parameter histograms gated on the appropriate populations created in Figure 1 to enumerate CD15+, CD11b+, CD16+, and CD14+ (see Figures 4a-4c).
   b. The table below indicates the quadrants and histograms used to calculate each population.

Population	Quadrants	Histogram Figure #	
		Gated on GR	Gated on MO
CD15+	2 + 4	4b	
CD11b+	1 + 2	4b	
CD16+	1 + 2	4c	
CD14+	1 + 2		4a

8. For Solastra ML2 HLA-DR-FITC/CD56-PE/CD34-ECD/CD117-PC5.5/CD45-PC7:
   a. Create dual parameter histograms gated on the appropriate populations created in Figure 1 to enumerate HLA-DR+, CD56+, CD34+ and CD117+ (see Figures 5a-5e).
   b. The table below indicates the quadrants and histograms used to calculate each population.

Population	Quadrants	Histogram Figure #		
		Gated on LY	Gated on MO	Gated on CD45^dim^/SS^low^
HLA-DR+	2 + 4		5b	5c
CD56+	1 + 2	5a		5c
CD34+	1 + 2			5d
CD117+	1 + 2			5e

9. For Solastra ML3 CD7-FITC/CD13-PE/CD34-ECD/CD33-PC5.5/CD45-PC7:
   a. Create dual parameter histograms gated on the appropriate populations created in Figure 1 to enumerate CD7+, CD13+, CD34+ and CD33+ (see Figures 6a-6f).
   b. The table below indicates the quadrants and histograms used to calculate each population.

Population	Quadrants	Histogram Figure #			
		Gated on LY	Gated on MO	Gated on GR	Gated on CD45^dim^/SS^low^
CD7+	2 + 4	6f			6c
CD13+	2 + 4			6b	6d
CD34+	1 + 2				6e
CD33+	2 + 4		6a		6e

10. Use the negatively stained populations for placement of the quadstat or region positive boundaries (see Figures 4-6).

    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.

Figure 4a-4c: Dual parameter histograms from a normal bone marrow specimen for CD16-ECD vs. CD14-PC5.5, CD15-FITC vs. CD11b-PE and CD11b-PE vs. CD16-ECD.

Figure 4a: Tube ML1 gated on monocytes (Gate B)

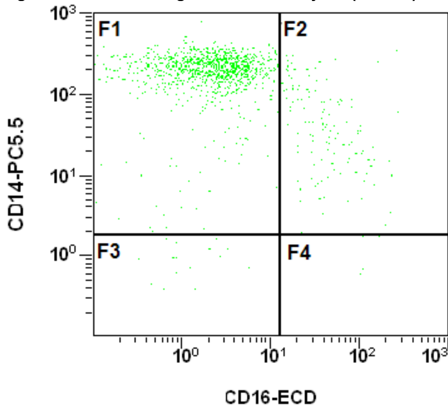


Figure 4b: Tube ML1 gated on granulocytes (Gate C)

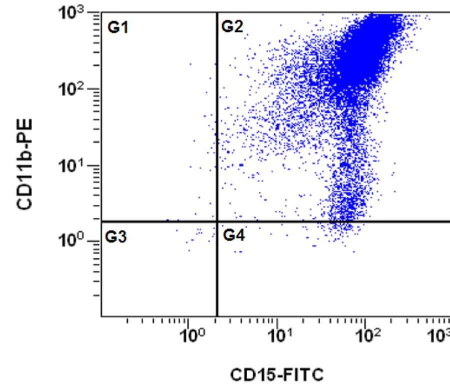


Figure 4c: Tube ML1 gated on granulocytes (Gate C)

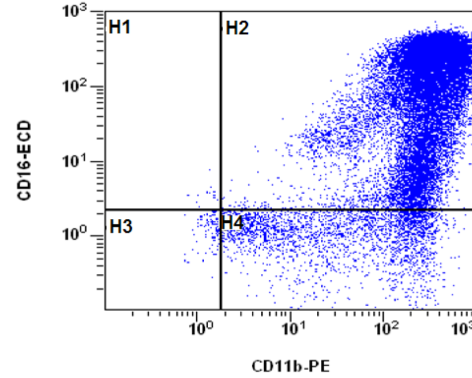


Figure 5a-5e: Dual parameter histograms from a normal bone marrow specimen for HLA-DR-FITC vs. CD56-PE, HLA-DR-FITC vs. CD34-ECD and CD34-ECD vs. CD117-PC5.5.

Figure 5a: Tube ML2 gated on lymphocytes (Gate A)

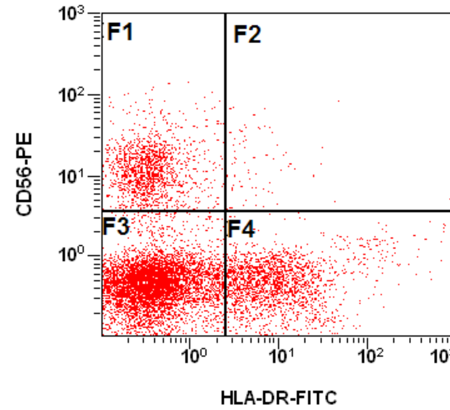


Figure 5b: Tube ML2 gated on monocytes (Gate B)

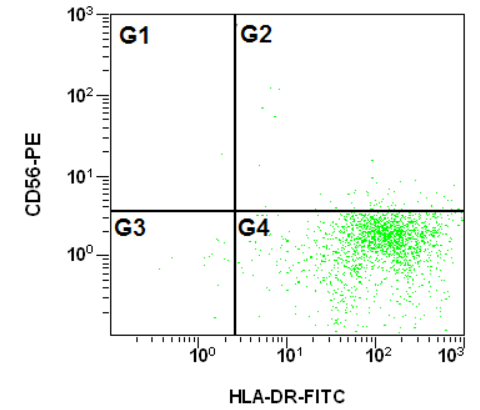


Figure 5c: Tube ML2 gated on CD45^dim^/SS^low^ (Gate D)

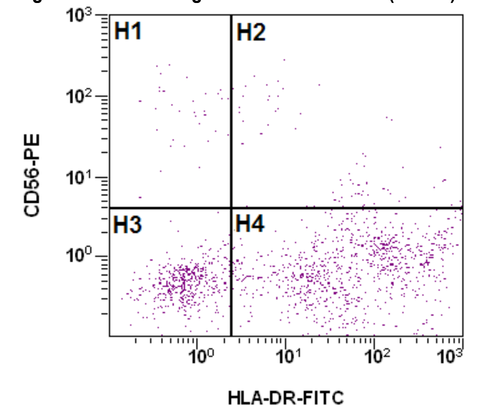


Figure 5d: Tube ML2 gated on CD45^dim^/SS^low^ (Gate D)

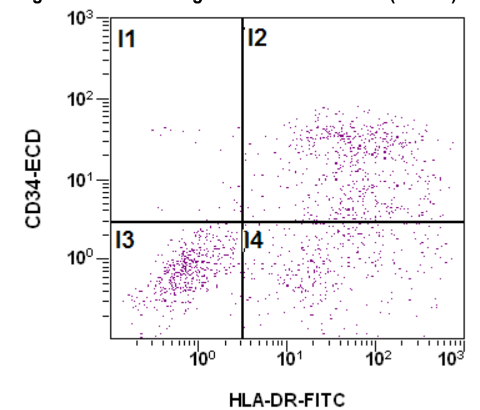


Figure 5e: Tube ML2 gated on CD45^dim^/SS^low^ (Gate D)

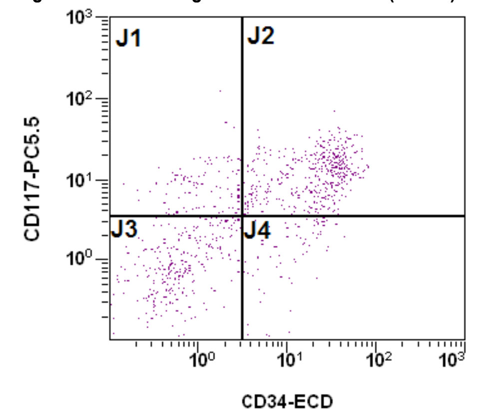


Figure 6a-6f: Dual parameter histograms from a normal bone marrow specimen for CD33-PC5.5 vs. CD34-ECD, CD13-PE vs. CD34-ECD and CD7-FITC vs. CD34-ECD.

Figure 6a: Tube ML3 gated on monocytes (Gate B)

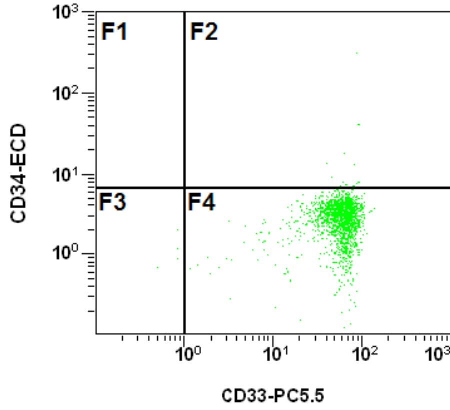


Figure 6b: Tube ML3 gated on granulocytes (Gate C)

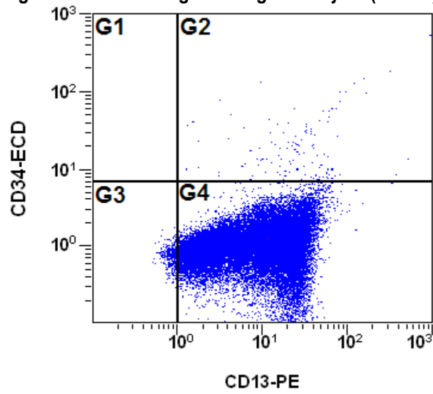


Figure 6c: Tube ML3 gated on CD45^{dim}/SS^{low} (Gate D)

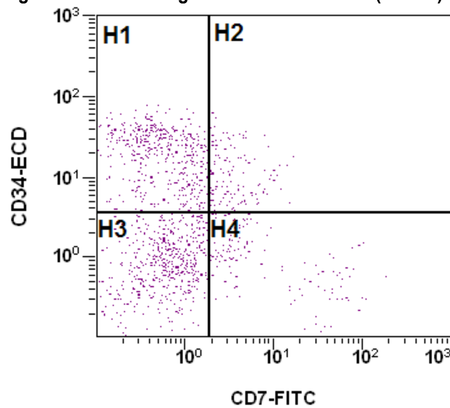


Figure 6d: Tube ML3 gated on CD45^{dim}/SS^{low} (Gate D)

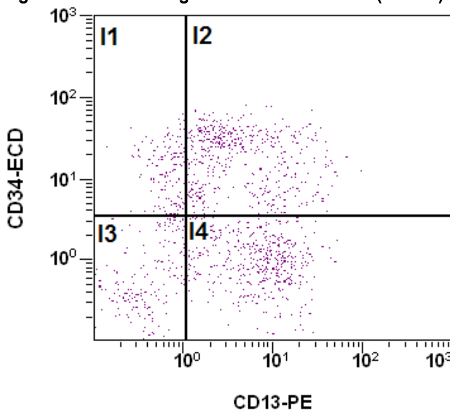


Figure 6e: Tube ML3 gated on CD45^{dim}/SS^{low} (Gate D)

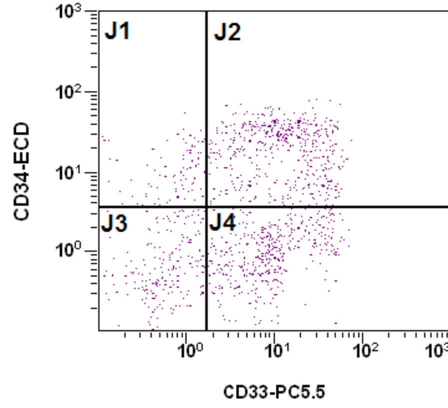


Figure 6f: Tube ML3 gated on lymphocytes (Gate A)

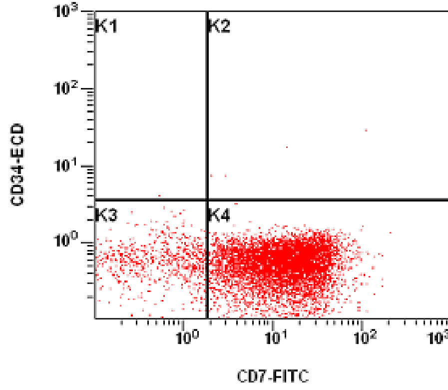
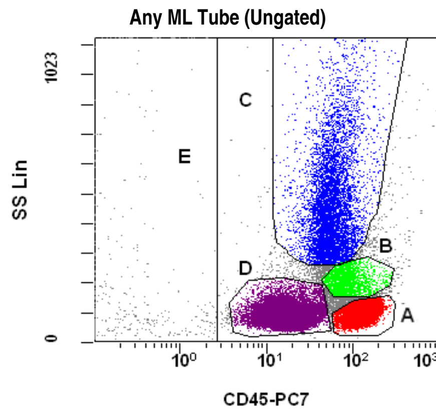


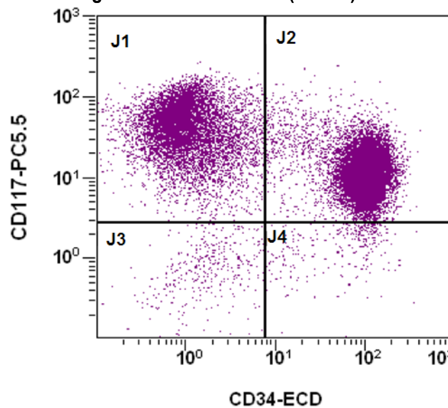
Figure 7: Representative examples of dual parameter histograms from an acute myeloid leukemia (AML) bone marrow sample for Solastra ML1, ML2 and ML3 reagents.

NOTE: Not all dual parameter histograms shown.

Any ML Tube (Ungated)



Tube ML2 gated on CD45^{dim}/SS^{low} (Gate D)



Tube ML3 gated on CD45^{dim}/SS^{low} (Gate D)

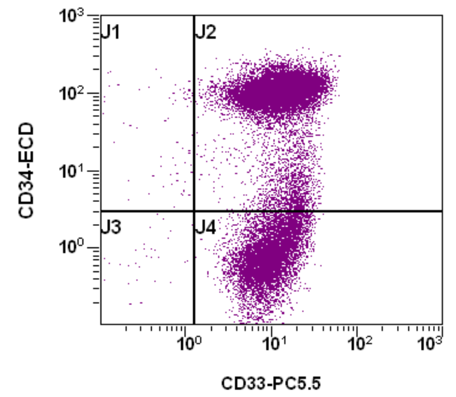
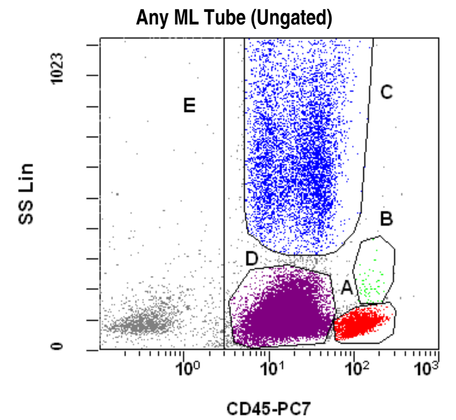


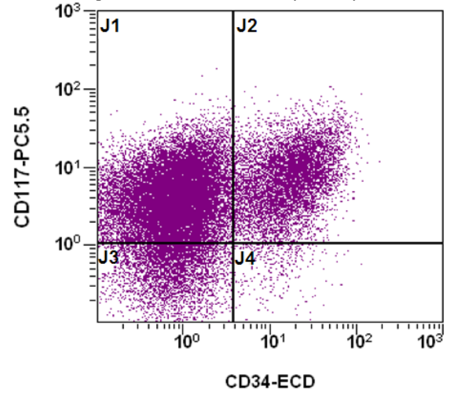
Figure 8: Representative examples of dual parameter histograms from an acute myeloid leukemia (AML) bone marrow sample for Solastra ML1, ML2 and ML3 reagents

NOTE: Not all dual parameter histograms shown.

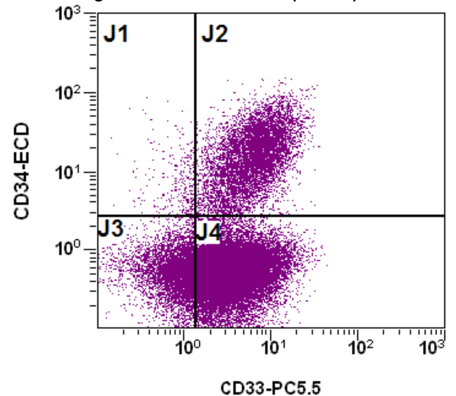
Any ML Tube (Ungated)



Tube ML2 gated on CD45^{dim}/SS^{low} (Gate D)



Tube ML3 gated on CD45^{dim}/SS^{low} (Gate D)



LIMITATIONS

1. EDTA collected specimens stained with Solastra ML1, Solastra ML2 and Solastra ML3 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.[11,17]
13. Cell aggregation may be observed in side scatter for lymph node specimens analyzed using the Myelomonocytic Lineage tube 2.

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 Myelomonocytic 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				
CD7+	130	81.01	65.80	92.79
CD56+	129	14.39	4.03	32.92
% Granulocytes				
CD11b+	130	99.66	97.58	100.00
CD15+	130	99.68	97.55	99.99
CD16+	130	95.48	84.59	99.68
CD13+	129	99.41	92.36	100.00
% Monocytes				
CD14+	129	96.94	90.18	99.46
CD33+	129	97.90	90.73	99.70
HLA-DR+	130	98.40	88.69	99.95

PERFORMANCE CHARACTERISTICS

The performance characteristics data presented below were 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]

CD15 antigen is expressed during hematopoiesis on myeloid progenitor cells. It is strongly expressed by mature cells of myelomonocytic lineage (neutrophils, eosinophils, monocytes, macrophages, mast cells). CD15 is not expressed on normal erythrocytes, platelets or lymphocytes.[18-19]

Expression of the CD11b chain on the cell surface requires the presence of the CD18 antigen (also known as β2 integrin chain). Together, these two subunits create the CD11b/CD18 integrin, one of the four integrin heterodimers that can be built by the association of CD18β chain with four distinctive CD11α chains. CD11b/CD18 is highly expressed on natural killer (NK) cells, neutrophils, monocytes and macrophages.

The CD16 antigen is the low affinity receptor of the G class immunoglobulins (IgG) (FcγRIII) expressed on NK cells, monocytes, macrophages and neutrophils.[20,21,45] The genetic heterogeneity of CD16 generates alternative membrane-anchored molecules. One is a transmembrane form (FcγRIIIA, 50-65 kD) expressed on NK cells, monocytes and macrophages.[45] The other is a glycosylphosphatidylinositol (GPI)-anchored form (FcγRIIIB, 48 kD) only expressed on neutrophils.[20,21]

The CD14 antigen is found on cells of the myelomonocytic lineage; strongly expressed on monocytes and macrophages and moderate to dim expression on peripheral blood neutrophils, and is present on pleural phagocytes and dendritic reticular cells. It is also weakly expressed by B lymphocytes, but is absent from T lymphocytes as well as from NK cells, erythrocytes and platelets.[22,23]

The HLA-DR antigen is found on antigen-presenting cells (APC), such as dendritic cells, B lymphocytes, monocytes, macrophages and thymic epithelial cells, but not on granulocytes, platelets, or red blood cells.[24,25] Stem Cells and hematopoietic progenitors express HLA-DR in the early stages of differentiation.[24,26] On T lymphocytes, the HLA-DR antigen is only expressed after activation.[27]

In hematopoietic cells, the 140 kD isoform of CD56 is expressed on a subpopulation of lymphocytes that demonstrate NK activity.[10-12] Virtually all of these cells capable of mediating non-TCR mediated cytotoxicity in peripheral blood express CD56.[28-30] This subpopulation consists of both NK cells (CD3-CD56+) and a small subset of T cells (CD3+CD56+).[10,12,30] CD56 is not expressed on other T or B lymphocyte, monocyte, granulocyte or erythrocyte populations.[30-32] CD56 may also be expressed at low levels on regenerating normal neutrophils and monocytes.[7,13-15]

The CD34 antigen is expressed on hematopoietic progenitor cells of all lineages as well as the pluripotent stem cells.[33-35] CD34 antigen expression is highest on the most primitive stem cells and is gradually lost as lineage committed progenitors differentiate.[33,35] The CD34 antigen is also present on capillary endothelial cells and on bone marrow stromal cells.[36,37]

CD117 antigen is the 145 kD proto-oncogene c-kit.[38] Within the hematopoietic compartment, the CD117 molecule is expressed on approximately 50 % of CD34+ progenitors engaged in erythrocytic,[39] myelomonocytic and megakaryocytic differentiation.[40] CD117 is also expressed on mast cells, but is not found on any other mature hematopoietic cells.

CD13 antigen is found on most cells of myeloid origin including neutrophils, eosinophils, basophils and monocytes from normal peripheral blood. It is absent from lymphocytes as well as from red blood cells and platelets. This molecule is found on the surface of committed progenitor cells defined as Granulocyte-Monocyte Colony Forming Units (GM-CFU) in normal bone marrow.[41-43]

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.[44] Mature B lymphocytes, cells of erythrocytic, myeloid, and megakaryocytic lineage do not normally express CD7.[44] CD7 may also be expressed in an aberrant manner in many cases of immature acute myeloid leukemia.[7,13]

CD33 antigen is strongly expressed on monocytes in peripheral blood, and weakly on circulating granulocytes. In the bone marrow it is found on hematopoietic progenitor cells of the myelomonocytic and erythroid lineage, but absent from cells of lymphoid origin.[43]

The antigen specificity of the CD11b, CD14, CD15, CD16 and CD45 monoclonal antibodies comprising the Solastra ML1 reagent, the CD34, CD45, CD56, CD117 and HLA-DR monoclonal antibodies comprising the Solastra ML2 reagent and the CD7, CD13, CD33, CD34 and CD45 monoclonal antibodies comprising the Solastra ML3 reagent has been previously established by the Human Leukocyte Differentiation Antigen Workshops.[8]

To assess cellular cross-reactivity, the CD15, CD11b, CD16, CD14, CD34, CD56, CD117, HLA-DR, CD13, CD7 and CD33 monoclonal antibodies comprising the Solastra ML1, Solastra ML2 and Solastra ML3 monoclonal antibody reagents were screened on normal human adult donor blood samples. Results consistently demonstrated that the CD15, CD11b, CD16, CD14, CD34, CD56, CD117, HLA-DR, CD13, CD7 and CD33 monoclonal antibodies reacted specifically with the appropriate leukocyte populations.

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 Myelomonocytic 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)
CD15+	y=0.9998x + 0.0079	0.9997
CD11b+	y=0.9979x + 0.0093	0.9997
CD16+	y=1.0051x - 0.0056	0.9996
CD14+	y=0.9961x + 0.0072	0.9997
HLA-DR+	y=0.9932x - 0.0045	0.9991
CD56+	y=0.9859x - 0.0010	0.9990
CD34+	y=1.0007x + 0.0203	0.9983
CD117+	y=0.9934x + 0.0249	0.9976
CD7+	y=0.9901x + 0.0198	0.9985
CD13+	y=0.9942x + 0.0202	0.9986
CD33+	y=0.9925x + 0.0195	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 Myelomonocytic Lineage Reagent Kit and comparator reagents and methods was studied in normal and abnormal blood, bone marrow and single cells 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 CD14+, CD34+ and CD56+ percentages on leukocytes.

ACCURACY OF METHOD

Measurement	n	Mean ± SD	Min	Max
% CD14+ gated on Monocytes (B)				
Solastra CD14+ (ML1)	152	91.68 ±13.15	6.45	99.87
COULTER CLONE CD14 (My4)-RD1	152	91.50 ±12.82	4.02	100.00
% CD56+ gated on Lymphocytes (A)				
Solastra CD56+ (ML2)	174	15.67 ±9.82	2.25	52.97
tetraCHROME CD45/CD56/CD19/ CD3	174	15.70 ±9.99	2.04	56.60
% CD56+ gated on $CD45^{dim}/SS^{low}$ (D)				
Solastra CD56+ (ML2)	13	37.15 ±41.75	2.26	95.60
tetraCHROME CD45/CD56/CD19/ CD3	13	37.99 ±42.12	2.47	95.92
% CD34+ gated on $CD45^{dim}/SS^{low}$ (D)				
Solastra CD34+ (ML2)	32	53.90 ±29.75	2.24	98.81
Stem-Kit CD34+	32	53.93 ±29.56	2.47	99.38
Solastra CD34+ (ML3)	32	54.47 ±30.69	2.77	99.43
Stem-Kit CD34+	32	53.10 ±29.76	2.47	99.38

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
% CD15+ gated on Granulocytes (C)				
Solastra CD15+ (ML1) Subset of CD45+ granulocytes	203	56.10 ±24.09	2.06	92.47
tetraCHROME CD45/CD56/ CD19/CD3	203	58.70 ±24.53	2.06	96.95
% CD11b+ gated on Granulocytes (C)				
Solastra CD11b+ (ML1) Subset of CD45+ granulocytes	200	54.62 ±22.45	2.05	92.07
tetraCHROME CD45/CD56/ CD19/CD3	200	59.52 ±23.77	2.06	96.95
% CD16+ gated on Granulocytes (C)				
Solastra CD16+ (ML1) Subset of CD45+ granulocytes	199	46.64 ±21.17	2.87	86.82
tetraCHROME CD45/CD56/ CD19/CD3	199	59.81 ±23.48	5.57	96.95
% HLA-DR+ gated on Monocytes (B)				
Solastra HLA-DR+ (ML2) Subset of CD45+ monocytes	143	5.92 ±6.93	2.06	64.72
tetraCHROME CD45/CD56/ CD19/CD3	143	6.21 ±7.94	2.07	71.39
% HLA-DR gated on $CD45^{dim}/SS^{low}$ (D)				
Solastra HLA-DR+ (ML2) Subset of $CD45^{dim}/SS^{low}$	27	26.81 ±33.73	2.06	95.52
tetraCHROME CD45/CD56/ CD19/CD3	27	36.09 ±36.96	2.53	95.61
% CD117+ gated on $CD45^{dim}/SS^{low}$ (D)				
Solastra CD117+ (ML2) Subset of $CD45^{dim}/SS^{low}$	16	28.00 ±28.39	2.18	89.15
tetraCHROME CD45/CD56/ CD19/CD3	16	42.89 ±35.02	3.22	93.43
% CD7+ gated on Lymphocytes (A)				
Solastra CD7+ (ML3) Subset of CD2+	208	19.00 ±14.45	2.01	75.11
CYTO-STAT CD2/CD20	208	68.76 ±26.34	4.76	99.28
% CD7+ gated on $CD45^{dim}/SS^{low}$ (D)				
Solastra CD7+ (ML3) Subset of $CD45^{dim}/SS^{low}$	6	45.14 ±27.49	12.81	88.00
tetraCHROME CD45/CD56/ CD19/CD3	6	67.55 ±21.32	43.44	95.61

Measurement	n	Mean ± SD	Min	Max
% CD13+ gated on Granulocytes (C)				
Solastra CD13+ (ML3) Subset of CD45+ granulocytes	202	46.27 ±22.18	2.19	90.28
tetraCHROME CD45/CD56/ CD19/CD3	202	58.96 ±24.31 1	2.40	96.95
% CD13+ gated on $CD45^{dim}/SS^{low}$ (D)				
Solastra CD13+ (ML3) Subset of $CD45^{dim}/SS^{low}$	16	18.10 ±19.13	2.11	63.23
tetraCHROME CD45/CD56/ CD19/CD3	16	36.69 ±37.01	2.44	93.43
% CD33+ gated on Monocytes (B)				
Solastra CD33+ (ML3) Subset of CD45+ monocytes	157	5.96 ±7.69	2.00	70.54
tetraCHROME CD45/CD56/ CD19/CD3	157	5.98 ±7.63	2.06	71.39
% CD33+ gated on $CD45^{dim}/SS^{low}$ (D)				
Solastra CD33+ (ML3) Subset of $CD45^{dim}/SS^{low}$	25	25.744 ±29.68	2.06	85.77
tetraCHROME CD45/CD56/ CD19/CD3	25	37.28 ±35.38	2.02	95.61

PRECISION

Within Run Variability

The percent positive values were determined using IMMUNO-TROL, IMMUNO-TROL with Stem-Trol Control Cells, and IMMUNO-TROL with MO7E cell line, run in duplicate, twice each day for up to 20 days at 4 geographically diverse sites using the Solastra Myelomonocytic Lineage Kit reagents.

Measurement	Repeatability (CV %)	Mean
% Lymphocytes		
CD7+	1.59 %	73.88
CD56+	7.34 %	14.16
% Granulocytes		
CD11b+	2.34 %	97.26
CD15+	0.20 %	99.76
CD16+	0.56 %	94.56
CD13+	0.43 %	99.91
% Monocytes		
CD14+	0.91 %	89.35
CD33+	0.94 %	95.44
HLA-DR+	0.56 %	95.20
% Stem-Trol Population		
CD34+ (from ML2)	9.26 %	11.85
CD34+ (from ML3)	7.00 %	11.75
% MO7E Cell Line Population		
CD117+	9.23 %	13.89

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PRODUCT AVAILABILITY

Solastra Myelomonocytic Lineage Kit

REF A66288

Solastra ML1 CD15-FITC/CD11b-PE/CD16-ECD/ CD14-PC5.5/CD45-PC7 - 25 tests (0.5 mL)

Solastra ML2 HLA-DR-FITC/CD56-PE/CD34-ECD/ CD117-PC5.5/CD45-PC7 - 25 tests (0.5 mL)

Solastra ML3 CD7-FITC/CD13-PE/CD34-ECD/ CD33-PC5.5/CD45-PC7 - 25 tests (0.5 mL)

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Beckman Coulter, Inc.
250 S. Kraemer Blvd.
Brea, CA 92821
www.beckmancoulter.com

Beckman Coulter Ireland Inc.
Mervue Business Park,
Mervue, Galway,
Ireland (353 91 774068)

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