1. INTENDED USE
BD CD8 (Leu-2a) phycoerythrin (PE) is a single-color, direct immunofluorescence reagent for enumerating percentages of mature human suppressor/cytotoxic (CD8+) lymphocytes in erythrocyte-lysed whole blood (LWB) or peripheral blood mononuclear cell (PBMC) suspensions.

2. SUMMARY AND EXPLANATION
Human lymphocytes may be divided into three major populations based on their biologic function and cell-surface antigen expression: T lymphocytes, B lymphocytes, and NK (natural killer) lymphocytes. T lymphocytes participate in antigen-specific cell-mediated immunity and regulate the secretion of immunoglobulin by B lymphocytes. T lymphocytes may also be classified based on their functional properties as helper/inducer or suppressor/cytotoxic T lymphocytes.

Clinical Applications*
The CD8 (Leu-2a)1,2 antibody recognizes an antigen expressed primarily on suppressor/cytotoxic T lymphocytes, although a subset of NK lymphocytes is also CD8 positive.3 Suppressor/cytotoxic lymphocytes are a subset of T lymphocytes (CD3+) that are CD8 positive.

A percentage of suppressor/cytotoxic lymphocytes may lie outside the normal reference range in some autoimmune diseases4 and in certain immune reactions such as acute graft-versus-host-disease (GVHD)5 and transplant rejection.6 The relative percentage of the CD8-positive subset is elevated in many patients with

* Not all the studies in this section employed BD reagents.
either congenital or acquired immune deficiencies such as severe combined immunodeficiency (SCID)\textsuperscript{7} and acquired immune deficiency syndrome (AIDS).\textsuperscript{18} The relative percentage of the CD8-positive cell population is often decreased in active systemic lupus erythematosus (SLE), but can also be increased in SLE patients undergoing steroid therapy.\textsuperscript{9}

For detailed information, refer to the CD8 PE Reference Guide.

3. PRINCIPLES OF THE PROCEDURE

When monoclonal antibody reagents are added to human whole blood, the fluorochrome-labeled antibodies bind specifically to antigens on the surface of leucocytes. Monoclonal antibodies may be used to identify lymphocyte subpopulations.

An aliquot of the stained patient sample is introduced into the flow cytometer and passed in a narrow stream through the path of a laser beam. The stained cells fluoresce when excited by the laser beam, and the emitted light is collected and processed by the flow cytometer.

For detailed information, refer to the CD8 PE Reference Guide.

4. REAGENT

Reagent Provided, Sufficient for 50 or 100 Tests

The BD CD8 PE reagent, sufficient for 50 or 100 tests, is a murine monoclonal antibody provided in buffered saline with gelatin and 0.1% sodium azide. It contains PE-labeled CD8 (Leu-2a), clone SK1,\textsuperscript{2} for identification of the CD8 positive suppressor/cytotoxic lymphocyte subset.

The antibody is composed of mouse IgG\textsubscript{1} heavy chains and kappa light chains. The CD8 antigen is present on the human suppressor/cytotoxic T-lymphocyte subset,\textsuperscript{1,11} as well as on a subset of NK lymphocytes.\textsuperscript{3} The CD8 antigen is expressed on the 32-kilodalton (kDa) \(\alpha\)-subunit of a disulfide-linked dimolecular complex. The cytoplasmic domain of the \(\alpha\)-subunit of the CD8 antigen is associated with the protein tyrosine kinase p56\textsuperscript{ck}.\textsuperscript{12,13}

**Precautions**

- For in vitro diagnostic use.
- When stored at 2°C–8°C, the antibody reagent is stable until the expiration date shown on the label. Do not use after the expiration date.
- The antibody reagent should not be frozen or exposed to direct light during storage or during incubation with cells. Keep the reagent vial dry.
- Alteration in the appearance of the reagent, such as precipitation or discoloration, indicates instability or deterioration. In such cases, the reagent should not be used.
- The antibody reagents contain sodium azide as a preservative; however, care should be taken to avoid microbial contamination, which may cause erroneous results.

**WARNING** All biological specimens and materials coming into contact with them are considered biohazards. Handle as if...
capable of transmitting infection\textsuperscript{14,15} and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

Concentration values are listed in the following table:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration (\textmu g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8 PE</td>
<td>12.5</td>
</tr>
</tbody>
</table>

For detailed information, refer to the CD8 PE Reference Guide.

5. INSTRUMENT

The CD8 PE reagent is designed for use on a BD flow cytometer equipped with appropriate computer hardware, software, and gating electronics. The flow cytometer must be equipped to detect two-color fluorescence, forward scatter (FSC), and side scatter (SSC).

We recommend using BD LYSYS\textsuperscript{TM} II software, version 1.1 or later, for data acquisition and analysis.

All performance characteristics were obtained using the BD FACScan\textsuperscript{TM} flow cytometer. Other systems may have different characteristics and should be verified by the user.

For information on use of this reagent for research purposes with other BD FACS\textsuperscript{TM} brand instruments, contact the BD Customer Support Center.

For detailed information, refer to the CD8 PE Reference Guide.

6. SPECIMEN AND COLLECTION PREPARATION

Blood samples may be prepared for flow cytometric analysis by using either LWB or PBMC preparation procedures. However, LWB is the recommended procedure\textsuperscript{10} since PBMC preparations yield more technique-dependent results.\textsuperscript{16}

Collect blood aseptically by venipuncture\textsuperscript{16,17} into a sterile BD Vacutainer\textsuperscript{TM} EDTA blood collection tube. A minimum of 1 mL of whole blood is required for LWB cell preparation, and a minimum of 2 mL of whole blood is required for PBMC preparation. Blood should be stained within 6 hours of venipuncture for optimal results. Anticoagulated blood may be stored at room temperature (20°C–25°C) for up to 6 hours until ready for staining. Blood samples refrigerated prior to staining may give aberrant results.

A white blood cell count (WBC) and a differential white-cell count should be obtained from the same sample of whole blood before staining. Samples with counts greater than 9.8 x 10\textsuperscript{3} WBC/\mu L may be diluted with 1X phosphate-buffered saline (PBS) containing 0.1% sodium azide. For samples with counts less than 3.5 x 10\textsuperscript{3} WBC/\mu L, more blood may be needed and a separation procedure may be required to concentrate the cells.

Interfering Conditions

Previously fixed and stored patient specimens should not be used. Whole blood samples refrigerated prior to staining may give aberrant results. For optimal results, blood samples should be stained within 6 hours of venipuncture. Samples obtained from patients taking immunosuppressive drugs may yield poor resolution.\textsuperscript{18} The presence of blast cells may interfere with test results. In LWB preparations, the presence of unlysed or nucleated red blood cells (RBCs) may
interfere with test results. Hemolyzed samples should be rejected. Follow the collection tube manufacturer’s guidelines for the minimum volume of blood to be collected.

**CAUTION** Use standard precautions when obtaining, handling, and disposing of all human blood samples and potentially carcinogenic reagents.

### 7. PROCEDURE

**Reagent Provided**

See Reagent Provided, Sufficient for 50 or 100 Tests, and Precautions in Section 4, Reagent.

**Reagents and Materials Required But Not Provided**

- *BD Simultest™ Leucogate™* (CD45 FITC/CD14 PE) reagent, 1 mL, (Catalog No. 340040). For determining a lymphocyte analysis gate, refer to the *BD Simultest Leucogate instructions for use* (IFU), the *BD Monoclonal Antibodies Source Book*, Section 5.2, and the *BD LYSYS II Software User’s Guide*. Store at 2°C–8°C.
- *Mouse IgG1 PE Control (γ1 PE) (keyhole limpet hemocyanin–specific)* reagent, 2 mL, (Catalog No. 349043). Store at 2°C–8°C.
- *BD Calibrite™ beads* (Catalog No. 349502. For detailed information on use, refer to the *BD Calibrite Beads IFU*.
- *BD FACSFlow™ sheath fluid* (Catalog No. 342003) or equivalent.
  
  **CAUTION** Use only *BD FACSFlow sheath fluid diluent* to dilute *BD Calibrite beads*.
- Paraformaldehyde (1%) for cell fixation. Dissolve 1 g of paraformaldehyde in 100 mL of freshly prepared PBS by carefully heating at temperatures up to 56°C in a chemical fume hood. Adjust to pH 7.4 ±0.2 with either 0.1 mol/L NaOH or 0.1 mol/L HCl. Filter with a 0.45-µm filter. Store in glass at 2°C–8°C.

**Materials for LWB Cell Preparation**

- *BD FACS lysing solution* (10X), 100 mL, (Catalog No. 349202. Store at 2°C–25°C. For use, dilute 1:10 with room temperature (20°C–25°C) reagent-grade water. Store in a glass container at room temperature. The prepared solution is stable for 1 month at room temperature. See the *BD FACS Lysing Solution IFU*.
- Reagent-grade (both distilled and deionized) water.

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* Falcon is a registered trademark of Corning Incorporated.  
* Low-speed centrifuge (minimum speed 200g) with swinging-bucket rotor and 12 x 75-mm tube carriers.  
* Vacuum aspirator with trap.  
* Micropipettor with tips.  
* Phosphate-buffered saline (PBS) (1X) (Dulbecco’s modified, pH 7.2 ±0.2; 0.01 mol/L PO4; and 0.15 mol/L NaCl). This reagent does not contain calcium, magnesium, phenol red, or sodium azide. Filter PBS through a 0.2-µm filter before use. Store at 2°C–8°C.
* BD FACSFlow™ sheath fluid (Catalog No. 342003) or equivalent.
Materials for PBMC Preparation

- Ficoll-Paque® separation medium. Refer to the Ficoll-Paque IFU for materials and reagents required.
- Cell viability testing reagents and materials for the ethidium bromide and acridine orange method (refer to Viability Staining Using Ethidium Bromide and Acridine Orange, Section 2.3, in the BD Monoclonal Antibodies Source Book) or trypan blue reagent for the trypan blue exclusion method.20

Staining and Fixing the Cells

The staining procedures for LWB and PBMC are described below. In either procedure, use care to protect the tubes from direct light. Both procedures should be performed at room temperature (20°C–25°C). For LWB, use room temperature reagents. For PBMC, use reagents at 2°C–25°C. Refer to Precautions in Section 4, Reagent.

LWB Cell Preparation

Whole blood samples are first stained with BD Simultest Leucogate reagent (tube A), Mouse IgG1 PE Control reagent (tube B), and the CD8 PE (tube C) reagent. Diluted (1X) BD FACS lysing solution is then used to lyse RBCs following staining. See Interfering Conditions under Section 6, Specimen and Collection Preparation, and refer to the CD8 PE Reference Guide.

1. For each patient sample, label three 12 x 75-mm tubes A, B, and C. Also label each tube with the sample identification number.

2. Place 20 µL of BD Simultest Leucogate reagent into tube A, 5 µL of Mouse IgG1 PE Control reagent and 15 µL of PBS with 0.1% sodium azide into tube B, and 20 µL of the CD8 PE reagent into tube C.

3. For each sample tube, use a fresh micropipettor tip and carefully add 100 µL of well-mixed, anticoagulated whole blood into the bottom of each of the three labeled tubes. The recommended WBC concentration is 3.5 x 10^3 to 9.8 x 10^3 WBC/µL. Vortex thoroughly at low speed for 3 seconds and incubate for 15–30 minutes at room temperature (20°C–25°C).

**NOTE** Protect samples from direct light during this incubation procedure and use care to prevent blood from running down the side of the tube. If whole blood remains on the side of the tube, it will not be stained with the reagent.

4. Dilute 10X BD FACS lysing solution to 1X following the instructions under Materials for LWB Cell Preparation in Section 7, Procedure. Add 2 mL of room temperature (20°C–25°C) 1X BD FACS lysing solution to each tube. Immediately vortex thoroughly at low speed for 3 seconds and incubate for 10–12 minutes at room temperature (20°C–25°C) in the dark. Do not exceed 12 minutes.

**NOTE** Avoid prolonged exposure of the cells to lytic reagents, which may cause white cell destruction. For detailed information, refer to the CD8 PE Reference Guide.

Ficoll-Paque® is a registered trademark of GE Healthcare.

Ficoll-Paque is a registered trademark of GE Healthcare.
5. Immediately after incubation, centrifuge tubes at 300 g for 5 minutes at room temperature (20°C–25°C).

6. Aspirate the supernatant leaving approximately 50 µL of residual fluid in the tube to avoid disturbing the pellet.

7. Vortex thoroughly at low speed to resuspend the cell pellet in the residual fluid and then add 2 mL of PBS with 0.1% sodium azide to each tube. Vortex thoroughly at low speed for 3 seconds. Centrifuge at 200 g for 5 minutes at room temperature (20°C–25°C).

8. Aspirate the supernatant leaving approximately 50 µL of residual fluid in the tube to avoid disturbing the pellet.

9. Vortex thoroughly at low speed to resuspend the cell pellet in the residual fluid and then add 0.5 mL of 1% paraformaldehyde to each tube. Vortex thoroughly at low speed for 3 seconds. Make sure that the cells are well mixed with the fixing solution.

10. The cells are now ready to be analyzed on the flow cytometer. Cap or cover the prepared tubes and store at 2°C–8°C in the dark until flow cytometric analysis. Analyze the fixed cells within 24 hours after staining. Vortex the cells thoroughly (at low speed) to reduce aggregation before running them through the flow cytometer.

PBMC Preparation

Peripheral blood samples are first separated using Ficoll-Paque. The PBMC suspension is then stained with BD Simultest Leucogate reagent (tube A), Mouse IgG1 PE Control reagent (tube B), and the CD8 PE (tube C) reagent. See Interfering Conditions under Section 6, Specimen and Collection Preparation.

1. Refer to the Ficoll-Paque IFU for instructions on performing mononuclear cell separation.

2. After the cells have been separated, determine the viability of the cells by staining with ethidium bromide and acridine orange (refer to Viability Staining Using Ethidium Bromide and Acridine Orange, Section 2.3, in the BD Monoclonal Antibodies Source Book) or by using trypan blue exclusion. Greater than 90% of the cells should be viable for the sample to be acceptable for use.

3. The cell suspension must be adjusted to a concentration of 1.5 to 2.5 x 10^7 cells/mL with PBS containing 0.1% sodium azide. Count cells using a microscope and hemacytometer or a hematology cell counter.

4. For each patient sample, label three 12 x 75-mm tubes A, B, and C. Also label each tube with the sample identification number.

5. Place 20 µL of BD Simultest Leucogate reagent into tube A, 5 µL of Mouse IgG1 PE Control reagent and 15 µL of PBS with 0.1% sodium azide into tube B, and 20 µL of the CD8 PE reagent into tube C.

6. For each sample tube, use a fresh micropipettor tip and carefully add 50 µL of mononuclear cell suspension into the bottom of each of the three labeled tubes. Vortex thoroughly at low speed for 3 seconds and incubate for 15–30 minutes at room temperature (20°C–25°C).

NOTE Protect samples from direct light during this incubation procedure.
and use care to prevent the cell suspension from running down the side of the tube. If cells remain on the side of the tube, they will not be stained with the reagent.

7. Add 2 mL of PBS with 0.1% sodium azide to each tube and vortex thoroughly at low speed for 3 seconds. Centrifuge tubes at 300g for 5 minutes at room temperature (20°C–25°C).

8. Aspirate the supernatant leaving approximately 50 µL of residual fluid in the tube to avoid disturbing the pellet.

9. Vortex thoroughly at low speed to resuspend the cell pellet in the residual fluid and then add 0.5 mL of 1% paraformaldehyde to each tube. Vortex thoroughly at low speed for 3 seconds. Make sure that the cells are well mixed with the fixing solution.

10. The cells are now ready to be analyzed on the flow cytometer. Cap or cover the prepared tubes and store at 2°C–8°C in the dark until flow cytometric analysis. Analyze the fixed cells within 24 hours after staining. Vortex the cells thoroughly (at low speed) to reduce aggregation before running them through the flow cytometer.

Flow Cytometry
Follow the BD instructions for two-color flow cytometric analysis (for BD Simultest Leucogate reagent) and refer to the CD8 PE Reference Guide.

See Figure 1, Figure 2, and Figure 3 for representative data.

Figure 1 shows the Leucogate lymphocyte analysis gate (tube A), Figure 2 shows the fluorescence markers set on the Control reagent (tube B), and Figure 3 shows the CD8+ suppressor/cytotoxic lymphocyte population present in tube C.

Figure 1. BD FACSscan dot plot (tube A) of a BD Simultest Leucogate-stained LWB sample from a hematologically normal female adult. The dot plot shows forward scatter (FSC) versus side scatter (SSC). The lymphocyte gate (1) is displayed, separating lymphocytes (2) from monocytes (3), granulocytes (4), and debris (5). The gate was drawn using BD Simultest Leucogate reagent.

Figure 2. BD FACSscan histogram (tube B) of an LWB sample, from same donor as in Figure 1, stained with Mouse IgG1 PE Control reagent. The figure displays fluorescence 2 (FL2) versus number of events (NoE), and the fluorescence marker (M1 marker) separates dimly stained events (interval [M0,M1]) from brightly stained events (interval [M1,M2]) in the lymphocyte gate. The marker is set at the lowest point where the
Mouse IgG1 PE negative control peak returns to baseline.

The values for the percent of gated events are shown in Table 1.

Figure 3  BD FACScan LWB histogram, from the same donor as in Figure 1, displaying FL2 versus number of events (NoE) for the CD8 PE reagent (tube C). The markers were set with the Control reagent (tube B).

Table 1  Values for percent of gated events (Figure 3)

<table>
<thead>
<tr>
<th>No.</th>
<th>Population</th>
<th>% Gated Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CD8+ events [M1,M2]</td>
<td>28.72</td>
</tr>
<tr>
<td>2</td>
<td>CD8- events [M0,M1]</td>
<td>71.28</td>
</tr>
</tbody>
</table>

For detailed information, refer to the CD8 PE Reference Guide.

Quality Control
For optimal results, we recommend using BD Calibrite beads and AutoCOMP™ software for setting the photomultiplier tube (PMT) voltages, setting the fluorescence compensation, and checking instrument sensitivity prior to use of BD LYSYS II software and CD8 PE reagent on the BD FACScan flow cytometer. Refer to the BD FACScan User's Guide and the BD LYSYS II Software User's Guide for information on optimizing the flow cytometer prior to analyzing patient samples.
We recommend that a control sample from a normal adult subject be run daily to optimize instrument settings and as a quality control check of the system. Correct results for a hematologically normal adult are previously illustrated in Figure 1, Figure 2, and Figure 3.

Mouse IgG1 Control reagent is run with each patient sample to set fluorescence markers between negative and positively stained lymphocyte clusters and to detect the presence of nonspecific staining that would indicate erroneous patient results. Visual inspection of the histogram obtained for tube C is necessary to ensure that fluorescence markers are correctly set and that there is minimal nonspecific staining. If poor separation between negative and positive populations is seen on visual inspection of the histogram for the normal control, nonspecific staining or incomplete staining can be inferred and the run should be rejected.

Use the following criteria for inspection of the dot plots, contour plots, or histograms obtained for each sample to evaluate the quality of the data obtained.

- The operator should reject the results if one or more of the following conditions is observed for the normal control: no separation between cellular populations; too few lymphocytes (less than 500); excessive RBC or nucleated RBC contamination and debris (greater than 10%), excessive monocyte (greater than 3%) or granulocyte (greater than 6%) contamination of the lymphocyte gate, or total lymphocytes within the gate is less than 95% of total lymphocytes in the sample.
- If there is no obvious reason for the normal control to fail, a sample from another normal control should be stained and run and the entire staining procedure repeated on all subsequent samples.
- In LWB preparations, samples with nucleated RBCs may contain too much debris because of incomplete lysis of nucleated erythrocytes with BD FACS lysing solution. Too much debris may also occur when assaying blood samples from patients with certain hematologic disorders where red cells are difficult to lyse, as in myelofibrosis and spherocytosis. Nucleated erythrocytes will be counted as debris and, if debris exceeds 10%, the sample results should be rejected.
- Under certain conditions, CD8-stained lymphocytes may form cell aggregates.21 As a result, these aggregated cells (escapees) may not be included within the lymphocyte gate and a lower percentage of CD8+ lymphocyte events will be obtained (see The Escapee Phenomenon, Section 8.2, in the BD Monoclonal Antibodies Source Book and Limitations in the CD8 PE Reference Guide). For cell suspensions prepared by Ficoll-Paque separation, it is recommended that a protein-containing medium be used (see The Escapee Phenomenon, Section 8.2, in the BD Monoclonal Antibodies Source Book).

NOTE Determinations of percentages of CD8+ lymphocytes made on LWB should not be compared to determinations made on PBMCs. Each laboratory should establish its own reference range appropriate to its standard technique.

For detailed information, refer to the CD8 PE Reference Guide.
8. RESULTS
Percent Lymphocyte Conversion
When the Percent Lymphocyte Conversion computation is performed, the CD8+ lymphocyte subset is reported as a percentage of lymphocytes in the lymphocyte analysis gate. If the computation is not performed, results will be reported as a percentage of the gated events. For detailed information, refer to the CD8 PE Reference Guide.

Absolute Counts
An absolute cell count can be computed if a WBC and the lymphocyte percentage from an independent differential white-cell count are obtained using standard laboratory procedures. For instructions on how to calculate absolute counts, refer to the CD8 PE Reference Guide.

9. LIMITATIONS
- Laboratories must establish their own normal reference ranges for the CD8 PE assay parameters, which may be affected by sex of patient, age of patient, and preparative technique. Race of patient may also have an effect, although sufficient data is not available to establish this. Age, sex, clinical status, and race of subjects should be known when a reference range is determined.
- If the results are to be expressed in absolute counts, an independent differential white-cell count and a WBC must also be run on the same sample of blood. The precision of the absolute count result obtained will depend on the precision for determining the percent of CD8+ lymphocytes and the precision for the WBC and the differential white-count assays. Absolute count values of lymphocyte subsets may not be comparable across laboratories because of variations in methods for determining white blood cell counts and/or white blood differential values.
- The CD8+ lymphocyte value derived from the CD8 PE reagent may be different from the value determined with the BD Simultest CD3/CD8 reagent, which reports suppressor/cytotoxic T lymphocytes that are positive for both CD3 and CD8, thereby eliminating CD3−CD8+ NK lymphocytes. A two-color analysis using a specific pan T-lymphocyte marker (for example, BD Simultest CD3/CD8 reagent) is recommended for identifying the suppressor/cytotoxic T-lymphocyte subset.16
- Abnormal states of health are not always represented by abnormal percentages of CD8+ suppressor/cytotoxic lymphocytes. That is, an individual who may be in an abnormal state of health may exhibit the same CD8+ lymphocyte percentages as a healthy individual. Results from the use of the CD8 PE reagent must be used in conjunction with other information available from the clinical evaluation and from additional independent diagnostic procedures, and interpreted by a medically qualified diagnostician to establish presence or absence of specific disease states.
- The CD8 PE reagent is not intended for screening samples for the presence of leukemic cells or for use in phenotyping samples from leukemia patients. The presence of blast cells may not allow the operator to set an adequate lymphocyte analysis gate using BD Simultest Leucogate reagent.
• PBMC specimens should have viability greater than 90%. Dead cells may stain nonspecifically. Prolonged contact of mononuclear cells with some lymphocyte separation media may reduce cell viability. Refer to the manufacturer’s instructions for information.

For detailed information, refer to the CD8 PE Reference Guide.

10. EXPECTED VALUES

Leucocyte Subsets
BD has investigated the normal reference ranges for CD8 (Leu-2a) PE reagent in normal male and female subjects using the BD FACScan flow cytometer at six clinical sites (five European sites and one US site). The formulation tested was BD Simultest IMK Plus CD4 (Leu-3a) FITC/CD8 (Leu-2a) PE reagent. Values of CD8+ lymphocytes obtained with CD8 PE reagent were equivalent to those obtained with BD Simultest CD4/CD8 reagent. The expected normal reference ranges of CD8+ suppressor/cytotoxic lymphocytes are shown in Table 2.22

Table 2 Representative reference ranges (LWB) CD8 PE reagent assay parameters in hematologically normal adults as percentages of total gated lymphocytes (converted) (data pooled from six clinical sites)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sex</th>
<th>Age</th>
<th>n</th>
<th>Mean</th>
<th>95% Range*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suppressor/cytotoxic lymphocytes</td>
<td>Both</td>
<td>20–70</td>
<td>304</td>
<td>33.4</td>
<td>18.9–47.9</td>
</tr>
</tbody>
</table>

Table 3 Within-sample reproducibility for CD8 PE reagent assay parameters (LWB) (five normal subjects and five abnormal subjects) as percentages of lymphocytes (converted)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5</td>
<td>Suppressor/cytotoxic lymphocytes</td>
<td>34.8</td>
<td>1.3</td>
<td>3.6</td>
<td>15</td>
</tr>
<tr>
<td>Abnormal</td>
<td>5</td>
<td>Suppressor/cytotoxic lymphocytes</td>
<td>65.5</td>
<td>2.6</td>
<td>3.7</td>
<td>15</td>
</tr>
</tbody>
</table>

NOTE Adult reference ranges should not be used with pediatric blood samples.

Race may also be a variable in the establishment of normal reference ranges,23 although insufficient data was collected by BD to determine this. Expected normal values may vary depending upon age, sex, race of patient, or cell preparation method. Because of these differences, each laboratory should establish its own normal reference range for each parameter.

Absolute Counts
For information on absolute counts and a representative reference range, refer to the CD8 PE Reference Guide.

11. PERFORMANCE

Performance of the CD8 PE reagent was established by testing at either one US clinical site or at BD laboratories in San Jose, California, or both.

Within-Sample Reproducibility
Table 3 and Table 4 show the average within-sample reproducibility obtained for both LWB and PBMC samples.

Table 3 Within-sample reproducibility for CD8 PE reagent assay parameters (LWB) (five normal subjects and five abnormal subjects) as percentages of lymphocytes (converted)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5</td>
<td>Suppressor/cytotoxic lymphocytes</td>
<td>34.8</td>
<td>1.3</td>
<td>3.6</td>
<td>15</td>
</tr>
<tr>
<td>Abnormal</td>
<td>5</td>
<td>Suppressor/cytotoxic lymphocytes</td>
<td>65.5</td>
<td>2.6</td>
<td>3.7</td>
<td>15</td>
</tr>
</tbody>
</table>

NOTE Adult reference ranges should not be used with pediatric blood samples.
Between-Instrument Reproducibility

Table 5 and Table 6 show the between-instrument reproducibility results.

### Table 5 Between-instrument reproducibility for CD8 PE reagent assay parameters (LWB) (ten normal subjects and three instruments) as percentages of lymphocytes (converted)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Parameter</th>
<th>Mean±</th>
<th>SD</th>
<th>CV</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>Suppressor/cytotoxic lymphocytes</td>
<td>39.0</td>
<td>1.37</td>
<td>3.28</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 6 Between-instrument reproducibility for CD8 PE reagent assay parameters (PBMC) (five normal subjects and three instruments) as percentages of lymphocytes (converted)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Parameter</th>
<th>Mean±</th>
<th>SD</th>
<th>CV</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5</td>
<td>Suppressor/cytotoxic lymphocytes</td>
<td>30.45</td>
<td>1.53</td>
<td>4.95</td>
<td>25</td>
</tr>
</tbody>
</table>

### CD8 PE Reagent versus Comparative Method

A summary of the results is presented in Table 7.

### Table 7 CD8 PE reagent versus comparative method using LWB and the FACScan

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Slope</th>
<th>Intercept</th>
<th>r</th>
<th>n²</th>
<th>Range of Data (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suppressor/cytotoxic lymphocytes</td>
<td>0.93</td>
<td>7.05</td>
<td>0.93</td>
<td>31</td>
<td>25–80</td>
</tr>
</tbody>
</table>

Stability of Stained Cell Preparations

We recommend analyzing samples within 24 hours of staining.

Cross-Reactivity

The CD8 (Leu-2a) antibody reacts with NK lymphocytes as well as with suppressor/cytotoxic T lymphocytes.

Linearity-Recovery

For an LWB sample, the normal range is 3.5 x 10³ to 9.8 x 10³ WBC/µL. Results are expected to be linear from 3.5 x 10³ to 9.8 x 10³ WBC/µL. For PBMCs, optimal performance is assured at 0.75 x 10⁸ to 1.25 x 10⁹ WBC/sample. PBMC concentration should be adjusted to 1.5 x 10⁷ to 2.5 x 10⁷ cells/mL.

For detailed information, refer to the CD8 PE Reference Guide.

**WARRANTY**

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REFERENCES

