Whole Blood  

Materials required, not provided  

1. Blood collection tubes containing anticoagulant (K2EDTA, heparin or ACD).  
2. Phosphate-buffered saline (PBS), 0.01 mol/L phosphate-buffered saline, pH 7.4.  
3. Flow cytometry using the whole blood method, and are presented in the following table. Values are expressed as a percent of the total lymphocyte count and are intended as representative values only. Each laboratory must determine its own expected values from the local population of normal donors.

### EXPECTED VALUES

<table>
<thead>
<tr>
<th>Variable</th>
<th>Min</th>
<th>Max</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>%CD8+ T cells</td>
<td>150</td>
<td>117</td>
<td>33</td>
</tr>
</tbody>
</table>

**FLOW CYTOMETRIC ANALYSIS**  

1. Analyze samples on a flow cytometer within 48 hours. If lyzed and fixed samples are held more than 2 hours, store at 4°C in dark until analysis (17, 18).

### REPRESENTATIVE HISTOGRAMS

- **Negative control, IgG1/PPE, Dako Code X0926.**
- **Lymphocytes from peripheral blood with anti-CD8/RPE conjugated anti-CD45/FITC and anti-CD14/FITC (Dako Code FF107).**
- **Isotypic control, IgG1/RPE, Dako Code X0926.**
- **Lymphocytes from peripheral blood with anti-CD8/RPE, Dako Code X0806.**

### SPECIMEN COLLECTION AND HANDLING

Collect blood specimens by venipuncture and centrifugation (13) into evacuated tubes containing an appropriate anticoagulant, completely expelling the vacuum in the tubes. Mix the blood well with the anticoagulant to prevent clotting. At least 100 μL of whole blood is required for each test. Ideally, blood samples should be processed immediately after collection.

When using ACD or heparin anticoagulants, blood samples may be lyzed at 18°C to 22°C before testing for up to 30 hours after venipuncture. When using EDTA, blood samples should be processed within 24 hours (14, 15).

**SAMPLE PREPARATION**

Optimum concentration of white blood cells for each test is (1 ± 0.3) x 10⁷ cells/mL. For samples with a high white blood cell count, dilute samples with autologous plasma to obtain a concentration of cells approximately equal to 1 x 10⁷ cells/mL. For samples containing a low white blood cell count (less than 0 x 10⁷ cells/mL), concentrate by centrifuging blood at 18-22°C for 5 minutes at 500 x G. Draw off buffy coat with some red cells and plasma to ensure that all white blood cells are collected. Resuspend cells in autologous plasma and adjust white blood cell concentration to approximately 1 x 10⁷ cells/mL. Proceed with staining procedure.

**In Vitro Diagnostic Use**

### PRECAUTIONS

- **Never pipette reagents by mouth and avoid their contact with skin and mucous membranes.**
- **Incubation times or temperatures other than those specified may give erroneous results; any such change must be validated by the user.**
- **If a fixation step is not included with the erythrocyte lysing procedure, resuspend pellet in 0.5 mL of 0.05-1% paraformaldehyde (fixative) in PBS for 30 minutes (16).**
- **If a 5% paraffin wax solution (Sigma-Aldrich catalog No. P9781) is used for total cell harvesting, add 0.1 mL of PBS Stock Solution and cool down. Filter through a 0.2 μm filter. Store at 2°C for up to 3 weeks.**
- **Flow cytometry gating may result in the CD8+ T-cell and CD4+ T-cell populations not being gate together.**
- **Fluorescent antibody emits at 570 nm, which displays orange-red.**
- **The cytometer operating characteristics, each laboratory must determine its own expected values from the local population of normal donors.**
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2. Mix equal volumes of cell suspension and Trypan Blue solution.
3. Place a sample of the mixture in a hemocytometer chamber. Do not overfill or underfill the chamber. (Note: The cell count should be performed between 5 and 15 minutes after addition of the dye. Trypan Blue has a greater affinity for serum proteins than for cellular proteins. If the background is too dark, cells should be washed in protein-free medium by centrifugation.)

4. Wash once more with the isotonic medium and centrifuge at 200 x g for 10 minutes.
5. Resuspend cells in the isotonic medium. The cells can then be counted and evaluated for cell viability and further studies.

6. Mix additional samples for control to one for the Dako isotopic control, IgG1/RPE (Dako Code X0028) and for the Isotype control for Anti-CD8/RPE, DK25 (Dako Code R0806, IgG1/RPE).

Specificity of Anti-CD8/RPE

1. Analyze cells on a flow cytometer properly standardized and gated on lymphocytes according to the instrument operating manual.
2. Analyze on a flow cytometer within 48 hours.

8. Barber EK, Dev Dasgupta J, Schlossman SF, Trevillian JM, Rudd CE. The CD4 and CD8 antigens are coupled to a protein-tyrosine kinase (p56Lck) that phosphorylates the CD3 complex. Proc Natl Acad Sci USA 1989; 86:9577

QUALITY ASSURANCE

Peripheral blood from a normal, apparently healthy donor should be run as a positive control to ensure proper working conditions. Normal ranges should be established within a local laboratory for normal, apparently healthy donors. Interference in the counts of positive cells by monocytes and granulocytes with Fc receptor antibody binding may be reduced by proper flow cytometric gating on lymphocytes. An appropriate Dako isotopic negative control that is non-human reactive (IgG1/RPE. Dako Code X0028) is used to control for nonspecific antibody binding to lymphocytes in each patient sample. The brightly-stained lymphocyte population is measured in markers set to exclude the low level of nonspecific fluorescence.

Non-specific fluorescence above the background cut-off is usually limited to 1-2% in normal individuals. If the background level above the cut-off for any control sample is greater than 1-2%, the resulting data may be erroneous.

LIMITATIONS

Patients should be stained within 30 hours of collection for optimal results. Retain samples in the original sample tube at room temperature prior to staining and analysis. Do not refrigerate. Refrigerated samples or samples stored longer than 30 days may give erroneous results. To ensure maximum quality, analyze stained cells promptly.

Samples from certain patients may present special problems due to abnormal erythrocyte or lymphocyte populations that are a result of illness or drug usage. Blood samples from abnormal donors may not show abnormal values for the percentage of lymphocytes stained with a monoclonal antibody. Results obtained from flow cytometric analysis should be reviewed with results from other diagnostic procedures.

Accuracy obtained by flow cytometric procedures depend upon correct alignment and calibration of the laser, as well as proper gating and compensation procedures.

Peripheral blood mononuclear cells (PBMCs) obtained by means of density gradient separation may not have the same relative percentages of lymphocytes as whole blood preparations. For normal patients, this factor may not be significant. For patients with leukemia, the selective exclusion of specific lymphocyte subsets may affect the accuracy of the results.

LINEARITY

A test of the Anti-CD25/RPE, DK25, monoclonal antibody was performed to determine the linearity of the binding to the CD25 cluster determinant. J77e cell line suspended cells (positive control for the CD25 antibody) were mixed with Raji cell line suspended cells (negative control for the CD25 antibody) at several concentrations (12491-001).

References