1. INTENDED USE

BD Tritest™ Control, \( \gamma_1/\gamma_1/\text{CD45} \) fluorescein isothiocyanate (FITC)/\( \gamma_1 \) phycoerythrin (PE)/\( \text{CD45} \) peridinin chlorophyll (PerCP), is a three-color direct immunofluorescence reagent used as an isotype (negative) control for flow cytometric immunophenotyping of erythrocyte-lysed whole blood with BD Tritest reagents on the BD family of flow cytometers. The BD Tritest Control is used to determine the unstained lymphocyte population on a fluorescence 1 (FL1) vs fluorescence 2 (FL2) display and to determine any nonantigen-specific antibody binding (nonspecific staining) present, particularly that caused by Fc receptors.\(^1\)\(^2\)

A BD Tritest Control is for in vitro diagnostic use with BD in vitro diagnostic BD Tritest reagents.

2. SUMMARY AND EXPLANATION

The BD Tritest Control \( \gamma_1/\gamma_1/\text{CD45} \) three-color reagent allows you to create an analysis gate to include \( \text{CD45} \)-positive lymphocytes in an FL3 (\( \text{CD45} \) PerCP) vs SSC display (see Figure 1).\(^2\)\(^3\) Once the lymphocyte population has been established, the BD Tritest Control is used to determine any nonspecific staining. Use the control to set quadrant markers on the FL1 vs FL2 display. Quadrant markers characterize cells according to the following properties: quadrant 1 (Q1) (upper left), PE-positive nonspecific staining; Q2 (upper right), PE- and FITC-positive nonspecific staining; Q3 (lower left), PE- and FITC-negative (unstained); and Q4 (lower right), FITC-positive nonspecific staining. The quadrant markers that show the boundaries of background and positive immunofluorescence from a
hematologically normal patient are shown in Figure 1.

**Figure 1** Lysed whole blood (LWB) sample, from a hematologically normal patient, stained with BD Tritest Control and analyzed on a BD FACScan flow cytometer.

**NOTE** The lymphocyte gate is drawn on an FL3 vs SSC dot plot. Fluorescence analysis is performed on an FL1 vs FL2 dot plot.

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**Clinical Applications**

The BD Tritest Control \(\gamma_1/\gamma_1/CD45\) reagent is used as a negative control for immunofluorescence staining of peripheral blood with BD Tritest reagents.

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**3. PRINCIPLES OF THE PROCEDURE**

When whole blood is added to the reagent, the fluorochrome-labeled antibodies in the reagent bind specifically to leukocyte surface antigens. If the reagent does not contain antibodies that are specific to the cell surface antigens, only nonspecific binding occurs.

BD FACSTM lysing solution is added to lyse erythrocytes before the sample is acquired on the flow cytometer. During acquisition, the cells travel past the laser beam and scatter the laser light. The stained cells fluoresce. These scatter and fluorescence signals, detected by the instrument, provide information about the cell’s size, internal complexity, and relative fluorescence intensity. BD Tritest reagents employ fluorescence triggering, allowing direct fluorescence gating of the lymphocyte population.\(^{2,3}\) This may reduce contamination of unlysed or nucleated red blood cells in the gate.

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**4. REAGENT**

**Reagent Provided, Sufficient for 50 Tests**

The BD Tritest Control \(\gamma_1/\gamma_1/CD45\) reagent, sufficient for 50 tests, is provided in 1 mL of buffered saline with bovine serum albumin and 0.1% sodium azide. It contains FITC- and PE-labeled IgG\(_1\), clone X40, and PerCP-labeled CD45, clone 2D1.\(^4\)

Mouse \(\gamma_1\) (IgG\(_1\)) recognizes a keyhole limpet hemocyanin (KLH) antigen not expressed on human cells. CD45 is
present on all human leucocytes and recognizes a 180–220-kilodalton (kDa) human leucocyte antigen that is a member of the leucocyte common antigen (LCA) family.5  

Mouse γ1 and CD45 antibodies are composed of mouse γ1 heavy chains and kappa light chains.  

Precautions  
• For in vitro diagnostic use.  
• Store the reagent at 2°C–8°C to maintain stability. Do not use after expiration date shown on the label.  
• Do not freeze the reagent or expose it to direct light during storage or during incubation with cells. Keep the reagent vial dry.  
• Do not use the reagent if you observe any changes in appearance. Precipitation or discoloration indicates instability or deterioration.  
• The antibody reagent contains sodium azide as a preservative; however, care should be taken to avoid microbial contamination, which may cause erroneous results.  
• All biological specimens and materials coming into contact with them are considered biohazards. Handle as if capable of transmitting infection6,7 and dispose of with proper precautions in accordance with federal, state, and local regulations when disposing of all materials. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.  
• BD FACS lysing solution is required and contains diethylene glycol and formaldehyde. Refer to the BD FACS Lysing Solution instructions for use (IFU) for warnings.

Concentration values are listed in the following table:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1 FITC</td>
<td>2.0</td>
</tr>
<tr>
<td>IgG1 PE</td>
<td>2.5</td>
</tr>
<tr>
<td>CD45 PerCP</td>
<td>6.25</td>
</tr>
</tbody>
</table>

5. INSTRUMENT  
The BD Tritest Control γ1/γ1/CD45 reagent is designed for use on a flow cytometer equipped with appropriate computer hardware and software. The flow cytometer must be equipped to detect three-color fluorescence, forward scatter (FSC), and side scatter (SSC). We recommend using BD CellQuest™ software for data acquisition and analysis and a BD flow cytometer such as a BD FACSCalibur™, BD FACS sort™, or BD FACScan™.

6. SPECIMEN COLLECTION AND PREPARATION  
Collect blood aseptically by venipuncture1,8 into a sterile BD Vacutainer® EDTA blood collection tube. A minimum of 1 mL of whole blood is required for this procedure. Follow the collection tube manufacturer’s guidelines for the minimum volume of blood to be collected. Store anticoagulated blood at room temperature (20°C–25°C) until ready for staining. Stain blood within 24 hours of venipuncture.  
Obtain a white blood cell (WBC) count and a differential white-cell count from the same whole blood sample before staining. Samples with WBC counts greater than 35.9 x 10^3 WBC/µL or lymphocyte counts greater than 15.0 x 10^3 lymphocytes/µL may be diluted.
with 1X phosphate-buffered saline (PBS) containing 0.1% sodium azide.

Interfering Conditions
Do not use previously fixed and stored patient specimens. Whole blood samples refrigerated prior to staining may give aberrant results. Samples obtained from patients taking immunosuppressive drugs may yield poor resolution. Blast cells may interfere with test results. Hemolyzed samples should be rejected.

7. PROCEDURE

Reagent Provided
BD Tritest Control $\gamma_1$ FITC/$\gamma_1$ PE/CD45 PerCP

Reagents and Materials Required But Not Provided
- BD Calibrite™ beads (Catalog No. 349502).
- The appropriate BD Tritest reagent.
- BD FACS lysing solution (10X), 100 mL (Catalog No. 349202). Refer to the BD FACS Lysing Solution IFU for dilution instructions and warnings.
- Reagent-grade (both distilled and deionized) water.
- BD Vacutainer EDTA blood collection tubes or equivalent.
- Falcon® disposable 12 x 75-mm polystyrene test tubes or equivalent.
- Vortex mixer.
- Micropipettor with tips.
- Bulk pipette dispenser (450 µL) for dispensing BD FACS lysing solution.
- BD FACSFlow™ sheath fluid (Catalog No. 342003) or equivalent.

CAUTION Use only BD FACSFlow sheath fluid diluent to dilute BD Calibrite beads.

Staining the Cells
Stain whole blood samples within 24 hours of venipuncture using BD Tritest Control reagent (tube A), and the appropriate BD Tritest reagent (tube B), and any other BD Tritest reagents in the panel in additional tubes. Then use diluted (1X) BD FACS lysing solution to lyse red blood cells following staining. Use care to protect the tubes from direct light. Perform the procedure at room temperature (20°C–25°C). See Precautions in Section 4, Reagent, and see Interfering Conditions in Section 6, Specimen Collection and Preparation.

1. For each patient sample, label two 12 x 75-mm tubes, A and B. If there are other reagents in the panel, label additional tubes as required. Also label each tube with the sample identification number.

2. Pipette 20 µL of BD Tritest Control reagent into the bottom of tube A and 20 µL of the appropriate BD Tritest reagent into the bottom of tube B. Pipette any other reagents from the BD Tritest panel into additional tubes as required.

3. Pipette 50 µL of well-mixed anticoagulated whole blood into the bottom of each tube using a new tip for each tube. Vortex gently to mix.

NOTE Avoid smearing blood down the side of the tube. If whole blood remains on the side of the tube, it will not be stained with the reagent.

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4. Incubate for 15 minutes in the dark at room temperature (20°C–25°C).

5. Add 450 µL of 1X BD FACS lysing solution to each tube. Vortex gently and incubate for 15–30 minutes at room temperature in the dark. The cells are now ready to be analyzed on the flow cytometer.10

6. If samples are not to be analyzed immediately after preparation, store them in the dark at room temperature for up to 24 hours and mix thoroughly just prior to analysis.

**Flow Cytometry**

Analyze the stained samples within 24 hours after preparation. Vortex the cells thoroughly (at low speed) to reduce aggregation before running them on the flow cytometer.11 Acquire list-mode data using the appropriate software such as BD CellQuest.

Refer to the appropriate BD Tritest three-color application note for flow cytometric setup, acquisition, and analysis.

We recommend using BD Calibrite beads and the appropriate software such as BD FACSComp™ software, version 1.1 or later, for setting the photomultiplier tube (PMT) voltages, setting the fluorescence compensation, and checking instrument sensitivity prior to use.

**Quality Control**

Run a sample daily from a normal adult to optimize instrument settings and as a quality control check of the system.1,2 Visually inspect the FL3 vs SSC dot plot for adequate separation between populations.

Run the BD Tritest Control reagent with each patient sample1,2 to set an analysis gate on the bright CD45 (FL3) lymphocyte population and to set fluorescence markers to detect the presence of nonspecific staining. See Cross-Reactivity in Section 11, Performance Characteristics, for quality control limits on nonspecific staining. See Figure 1 for representative data from a hematologically normal adult.

Visually inspect the FL1 vs FL2 dot plot for the BD Tritest Control reagent (tube A). If the negative cluster is diffuse and smeared over the FL1 or FL2 intensity range, the marker may not be set correctly and results may be suspect.

If there is no obvious reason for the normal control to fail, stain and run a sample from another normal adult subject. Repeat the entire staining procedure on all subsequent samples.

**8. RESULTS**

**Nonantigen-Specific Antibody Binding (Nonspecific Staining)**

Events falling in Q1 will have only FL2 fluorescence, those in Q4 will have only FL1 fluorescence, and those in Q2 will have both FL1 and FL2 fluorescence. Events in Q3 have low fluorescence in FL1 and FL2, and are a measure of unstained lymphocytes and contaminating nonlymphocytes in the gate.

Visual inspection of the dot plot obtained for tube B (and any additional tubes) is necessary to ensure that fluorescence markers are correctly set and that there is minimal nonspecific staining.

**9. LIMITATIONS**

Although the determination of nonspecific staining is assessed from the BD Tritest
Control (tube A), you should examine the SSC vs FL3 and FL1 vs FL2 dot plots for any evidence of nonspecific staining in tube B and any additional BD Tritest tubes. Nonspecific staining may be indicated when there is poor separation between positive and unstained clusters on the FL1 vs FL2 dot plot. The presence of such nonspecific staining may reflect a change in the reagent or an error in the preparation of the samples. Such samples should be restained.

10. EXPECTED VALUES

For expected values obtained using the BD Tritest Control, refer to the specific BD in vitro diagnostic BD Tritest reagent IFU.

11. PERFORMANCE CHARACTERISTICS

For lymphocyte immunophenotyping performance data obtained using the BD Tritest control, refer to the specific in vitro diagnostic BD Tritest reagent IFU.

Stability

We recommend staining samples within 24 hours of draw, and analyzing samples within 24 hours of staining.

Cross-Reactivity

Although the BD Tritest Control reagent is designed to detect nonspecific staining, nonspecific staining due to the poor condition of cells may be observed with any antibody reagent. Nonspecific staining can be inferred if no clear separation between unstained and positive cells is seen on the FL1 vs FL2 dot plot or if more than 5% of the events for the BD Tritest Control (tube A) appear outside Q3.

Linearity

Linearity of response over a wide range of WBC and lymphocyte counts is determined for each BD in vitro diagnostic immunophenotyping reagent using BD Tritest Control in the assay procedure. Refer to the specific in vitro diagnostic BD Tritest reagent IFU for the usable WBC and lymphocyte range.

WARRANTY

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

THE PRODUCTS SOLD HERUNDER ARE WARRANTED ONLY TO CONFORM TO THE QUANTITY AND CONTENTS STATED ON THE LABEL OR IN THE PRODUCT LABELING AT THE TIME OF DELIVERY TO THE CUSTOMER. BD DISCLAIMS HEREBY ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY AND FITNESS FOR ANY PARTICULAR PURPOSE AND NONINFRINGEMENT. BD'S SOLE LIABILITY IS LIMITED TO EITHER REPLACEMENT OF THE PRODUCTS OR REFUND OF THE PURCHASE PRICE. BD IS NOT LIABLE FOR PROPERTY DAMAGE OR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING PERSONAL INJURY, OR ECONOMIC LOSS, CAUSED BY THE PRODUCT.

REFERENCES


