**1. INTENDED USE**

Anti-Lambda is intended for in vitro diagnostic use in the identification of cells expressing lambda light chains, using a BD FACS™ brand flow cytometer.

The flow cytometer must be equipped to detect light scatter and the appropriate fluorescence, and be equipped with appropriate analysis software (such as BD FACSDiva™, BD CellQuest™ Pro, or BD CellQuest™ software) for data acquisition and analysis. See your instrument user’s guide for instructions.

**Applications**

Expression of lambda light chains in the characterization of hematologic neoplasia.

**2. COMPOSITION**

Anti-Lambda, clone 1-155-2,* is derived from hybridization of mouse P3-X63-Ag8.653 myeloma cells with cells from BALB/cJ mice immunized with human IgA1-λ myeloma protein. Anti-Lambda is composed of mouse IgG1 heavy chains and lambda light chains.

The FITC reagent is supplied in phosphate-buffered saline (PBS) containing gelatin and 0.1% sodium azide. The PE reagent is supplied in buffer containing bovine serum albumin (BSA) and 0.1% sodium azide. Concentrations are listed in Table 1.

<table>
<thead>
<tr>
<th>Form</th>
<th>Amount provided</th>
<th>Concd (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC</td>
<td>12.5 µg in 1.0 mL of PBS</td>
<td>12.5</td>
</tr>
<tr>
<td>PE</td>
<td>12.5 µg in 1.0 mL of buffer</td>
<td>12.5</td>
</tr>
</tbody>
</table>

* This clone has not been submitted to any previous Workshop on Human Leucocyte Differentiation Antigens.
Antibody purity is as follows.

- FITC: ≤5% free fluorophore at bottling, as measured by size-exclusion chromatography (SEC)
- PE: ≤20% free fluorophore at bottling, as measured by SEC

3. STORAGE AND HANDLING

The antibody reagent is stable until the expiration date shown on the label when stored at 2°C–8°C. Do not use after the expiration date. Do not freeze the reagent or expose it to direct light during storage or incubation with cells. Keep the outside of the reagent vial dry.

Do not use the reagent if you observe any change in appearance. Precipitation or discoloration indicates instability or deterioration.

4. REAGENTS OR MATERIALS REQUIRED BUT NOT PROVIDED

- Falcon® disposable 12 x 75-mm polystyrene test tubes or equivalent
- Micro pipettor with tips
- Vortex mixer
- Centrifuge
- BD FACS™ lysing solution (10X) (Catalog No. 349202)
  For dilution instructions and warnings, see the instructions for use (IFU).
- BD CellWASH™ (Catalog No. 349524) or a wash buffer of PBS with 0.1% sodium azide
- BD CellFIX™ (Catalog No. 340181) or 1% paraformaldehyde solution in PBS with 0.1% sodium azide
  Store at 2°C–8°C in amber glass for up to 1 week.
- BD FACS brand flow cytometer
  See the appropriate instrument user’s guide for information.

5. SPECIMEN(S)

Reagents can be used for immunophenotyping by flow cytometry with a variety of specimen types, including peripheral blood, bone marrow aspirates or biopsies, and other body fluids or tissues. Each type of specimen can have different storage conditions and limitations that should be considered prior to collection and analysis.2,3

Samples with large numbers of nonviable cells can give erroneous results due to selective loss of populations and to increased nonspecific binding of antibodies to nonviable cells. Viability of samples should be assessed and a cut-off value established. A cut-off value of at least 80% viable cells has been suggested.2

WARNING

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection4,5 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.

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a. Conc = concentration

† Falcon is a registered trademark of Corning Incorporated.
6. PROCEDURE

1. To avoid serum interference when using this reagent, prewash the whole blood sample using at least 25 volumes excess 1X PBS with 0.1% sodium azide (48 mL of 1X PBS with sodium azide per 2 mL of whole blood to be washed). Mix well.

2. Pellet cells by centrifugation and resuspend in 1X PBS with 0.1% sodium azide to the original volume.

3. Add the appropriate volume of Anti-Lambda fluorochrome-conjugated monoclonal antibody to 100 µL of cell suspension in a 12 x 75-mm tube. See the vial label for volume.

4. Vortex gently and incubate 15 to 30 minutes in the dark at room temperature (20°C–25°C).

5. Add 2 mL of 1X BD FACS lysing solution.

6. Vortex gently and incubate for 10 minutes in the dark at room temperature.

7. Centrifuge at 300 g for 5 minutes. Remove the supernatant.

8. Add 2 to 3 mL of BD CellWASH solution (or wash buffer) and centrifuge at 200 g for 5 minutes. Remove the supernatant.

9. Add 0.5 mL of BD CellFIX solution (or 1% paraformaldehyde solution) and mix thoroughly. Store at 2°C–8°C until analyzed.

We recommend analyzing within 24 hours of staining.

Analytical Results

Abnormal numbers of cells expressing this antigen or aberrant expression levels of the antigen can be expected in some disease states. It is important to understand the normal expression pattern for this antigen and its relationship to expression of other relevant antigens to perform appropriate analysis.

Flow Cytometry

Vortex the cells thoroughly at low speed to reduce aggregation before running them on the flow cytometer. Acquire and analyze list-mode data using appropriate software. Before acquiring samples, adjust the threshold to minimize debris and ensure that populations of interest are included. Figure 1 displays representative data from pre-washed normal peripheral blood and gated on CD20+ B cells. Laser excitation is at 488 nm.
Internal Quality Control
To set photomultiplier tube (PMT) voltages, fluorescence compensation, and to check instrument sensitivity before acquisition, use one of the following combinations:

- For BD FACSCanto™ and BD FACSCanto™ II cytometer setup, use BD FACS™ 7-color setup beads and BD FACSComp™ software. See the IFU and instrument user's guide.
- For BD FACSCalibur™ cytometer setup, use BD Calibrite™ beads and BD FACSCalibur™ software. See the IFU and instrument user's guide.

We recommend running a control sample daily from a normal adult subject or a commercially available whole blood control to optimize instrument settings and as a quality control check of the system.7

7. PERFORMANCE CHARACTERISTICS

Specificity
The Anti-Lambda antibody is specific for lambda light chains of human immunoglobulins.8 Immunoglobulins bearing lambda light chains are present on approximately 40% of normal B lymphocytes and on Igλ+ leukemic cells.9-17 In serum, Anti-Lambda reacts with immunoglobulins bearing lambda light chains as well as free lambda light chains.

Sensitivity
Sensitivity is defined as resolution of the Anti-Lambda+ population from the Anti-Lambda− population. Sensitivity was measured by evaluating a range of antibody concentrations. Each concentration of reagent was tested on lymphocytes. The separation of Anti-Lambda+ from Anti-Lambda− was determined for each sample and averaged within each concentration. The bottled antibody concentration for each reagent provided optimum sensitivity in resolving the Anti-Lambda+ cells from the negative. See Table 1.

Reproducibility
Estimates of assay reproducibility were determined at BD Biosciences using peripheral whole blood samples stained in duplicate with the reagent and acquired on a BD FACSCalibur flow cytometer.
The components of reproducibility include instrument-to-instrument, operator-to-operator, run-to-run, day-to-day, and lot-to-lot (inter-assay precision). The average signal to noise (S/N) ratio, standard deviation (SD), and coefficients of variation (CVs), excluding the day-to-day component, are provided in Table 2.

When the day-to-day component was included, the reproducibility of the reagent was 86.1 for the averages with an SD of 33.9 and a %CV of 39.4 due to the degradation of the target molecule in whole blood. However, when the reproducibility of the assay for the percent positive (% positive) was determined, the inter-assay precision was acceptable (Table 3).

Repeatability
Estimates of assay repeatability were determined at BD Biosciences using peripheral whole blood samples stained in duplicate with the reagent and acquired on a BD FACSCalibur flow cytometer. The components of repeatability include tube-to-tube (intra-assay precision). The average S/N ratio, SD, and CVs are provided in Table 4.

8. LIMITATIONS
Conjugates with brighter fluorochromes (PE, APC) will give greater separation than those with other dyes (FITC, PerCP). When populations overlap, calculation of the percentage of cells positive for the marker can be affected by the choice of fluorochrome.

Use of monoclonal antibodies in patient treatment can interfere with recognition of target antigens by this reagent. This should be considered when analyzing samples from patients treated in this fashion. BD Biosciences has not characterized the effect of the presence of therapeutic antibodies on the performance of this reagent.

Single reagents can provide only limited information in the analysis of leukemias and lymphomas. Using combinations of reagents can provide more information than using the reagents individually. Multicolor analysis using relevant combinations of reagents is highly recommended.3

Since reagents can be used in different combinations, laboratories need to become familiar with the properties of each antibody in conjunction with other markers in normal and abnormal samples. Reagent performance data was collected typically with EDTA-treated blood.

<table>
<thead>
<tr>
<th>Table 2 Inter-assay precision of the assay without day-to-day component (S/N ratio)</th>
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</thead>
<tbody>
<tr>
<td>PE</td>
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</table>

<table>
<thead>
<tr>
<th>Table 3 Inter-assay precision of the assay with day-to-day component (% positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
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</table>

<table>
<thead>
<tr>
<th>Table 4 Intra-assay precision of the assay</th>
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<tbody>
<tr>
<td>PE</td>
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</tbody>
</table>

a. df = degree of freedom
Reagent performance can be affected by the use of other anticoagulants.

**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 HEREUNDER 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 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.

**TROUBLESHOOTING**

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor resolution</td>
<td>Cell interaction with other cells</td>
<td>Prepare and stain another sample.</td>
</tr>
<tr>
<td>between debris and</td>
<td>and platelets</td>
<td></td>
</tr>
<tr>
<td>lymphocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rough handling</td>
<td>Check cell viability; centrifuge</td>
<td>Cell concentration too high at staining step.</td>
</tr>
<tr>
<td>of cell preparation</td>
<td>cells at lower speed.</td>
<td></td>
</tr>
<tr>
<td>Inappropriate</td>
<td>Follow proper instrument setup</td>
<td>Cell concentration too high at staining step.</td>
</tr>
<tr>
<td>instrument settings</td>
<td>procedures; optimize instrument</td>
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</tr>
<tr>
<td></td>
<td>settings as required.</td>
<td></td>
</tr>
<tr>
<td>Staining dim or fading</td>
<td>Check and adjust cell concentration</td>
<td>Cell concentration too high at staining step.</td>
</tr>
<tr>
<td></td>
<td>or sample volume; stain with fresh</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sample.</td>
<td></td>
</tr>
<tr>
<td>Insufficient reagent</td>
<td>Repeat staining with increased</td>
<td>Insufficient reagent.</td>
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<tr>
<td></td>
<td>amount of antibody.</td>
<td></td>
</tr>
<tr>
<td>Cells not analyzed</td>
<td>Repeat staining with fresh sample.</td>
<td>Cells not analyzed within 24 hours of</td>
</tr>
<tr>
<td>within 24 hours of</td>
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<td>staining.</td>
</tr>
<tr>
<td>staining</td>
<td></td>
<td></td>
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<tr>
<td>Improper medium</td>
<td>Use sodium azide in staining and</td>
<td>Improper medium preparation (sodium azide</td>
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<tr>
<td>preparation (sodium azide</td>
<td>washing steps.</td>
<td>omitted).</td>
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</table>

**REFERENCES**


