1. INTENDED USE

QuantiBRITE™ CD20 is intended for in vitro diagnostic use in the identification of cells expressing CD20 antigen, using a 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 CellQuest™ or LYSYS™ II) for data acquisition and analysis. Refer to your instrument user’s guide for instructions.

Applications

Expression of CD20 in the characterization of hematologic neoplasia

2. COMPOSITION

CD20, clone L27, is derived from hybridization of mouse Sp2/0 myeloma cells with spleen cells from BALB/c mice immunized with the LB lymphoblastoid cell line. CD20 is composed of mouse IgG1 heavy chains and kappa light chains.

CD20 PE is supplied in phosphate-buffered saline (PBS) containing bovine serum albumin (BSA) and 0.1% sodium azide. Concentrations are listed in Table 1.

<table>
<thead>
<tr>
<th>PE</th>
<th>12.5 µg/mL of PBS</th>
<th>12.5 µg/mL</th>
</tr>
</thead>
</table>

Table 1. Bottling concentrations

WARNING: Sodium azide is harmful if swallowed (R22). Keep out of reach of children (S2). Keep away from food, drink, and animal feedingstuff (S13). Wear
suitable protective clothing (S36). If swallowed, seek medical advice immediately and show this container or label (S46). Contact with acids liberates very toxic gas (R32). Azide compounds should be flushed with large volumes of water during disposal to avoid deposits in lead or copper plumbing where explosive conditions can develop.

Antibody purity is as follows.

PE: ≥95% 1:1 PE:mAb ratio, as measured by size-exclusion chromatography (SEC)

3. STORAGE AND HANDLING
The antibody reagent is stable until the expiration date shown on the label when stored at 2° to 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
1. Falcon™ disposable 12 x 75-mm capped polystyrene test tubes (BD Catalog No. 352058) or equivalent
2. Micropipettor with tips (BD Electronic Pipette, BD Catalog No. 646539) or equivalent
3. Vortex mixer
4. FACS Lysing Solution (10X) (BD Catalog No. 349202). For dilution instructions and warnings, refer to the product insert.
5. QuantiBRITE PE Phycoerythrin Fluorescence Quantitation Kit (BD Catalog No. 340495). Refer to the product data sheet for instructions.
6. Centrifuge
7. CellWASH™ (BD Catalog No. 349524) or a wash buffer of phosphate-buffered saline (PBS) with 0.1% sodium azide.
8. CellFIX™ (BD Catalog No. 340181) or 1% paraformaldehyde solution in PBS with 0.1% sodium azide. Store at 2° to 8°C in amber glass for up to 1 week.

WARNING: Formaldehyde is harmful by inhalation, in contact with skin, and if swallowed (R20/21/22). It is irritating to eyes and skin (R36/38). Exposure can cause cancer. Possible risks of irreversible effects (R68). Can cause sensitization by skin contact (R43). Keep locked up and out of the reach of children (S1/2). Wear suitable protective clothing and gloves (S36/37). If swallowed, seek medical advice immediately and show the container or label (S46). Dispose of according to federal, state, and local regulations.

9. FACS brand flow cytometer. Refer to 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.

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.

WARNING: All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing and gloves. Fixation has been reported to inactivate HIV.

6. PROCEDURE

1. Add the appropriate volume of CD20 fluorochrome-conjugated monoclonal antibody to 100 μL of whole blood in a 12 x 75-mm tube. Refer to the appropriate vial label for volume.
2. Vortex gently and incubate for 15 to 30 minutes in the dark at room temperature (20° to 25°C).
3. Add 2 mL of 1X FACS Lysing Solution.
4. Vortex gently and incubate for 10 minutes in the dark at room temperature.
5. Centrifuge at 500 x g for 5 minutes. Remove the supernatant.
6. Add 2 to 3 mL of CellWASH (or wash buffer) and centrifuge at 500 x g for 5 minutes. Remove the supernatant.
7. Add 0.5 mL of CellFIX (or 1% paraformaldehyde solution) and mix thoroughly. Store at 2° to 8°C until analyzed. BD Biosciences recommends 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 in order to perform appropriate analysis.

Flow Cytometry

Vortex the cells thoroughly at low speed to reduce aggregation before running them on the flow cytometer. When ready to read samples, dilute a tube of fresh QuantiBRITE beads according to package instructions. Set up the instrument using FACSComp™ software, version 4.2, lyse/no-wash (LNW) setting and run the samples and the QuantiBRITE tube using the same settings. Before acquiring samples, adjust the threshold to minimize
debris and ensure populations of interest are included. Figure 1 displays representative data performed on peripheral blood and gated on lymphocytes. Laser excitation is at 488 nm.

**Figure 1** Representative data analyzed with a FACS brand flow cytometer

Analyze your data using Attractors™, version 3.1 or higher, or QuantiCALC™, automated quantitation analysis software that converts the FL2 axis to PE-conjugated–antibodies bound per cell (ABC) and reports statistics in CD20 PE ABC. Alternatively, you can perform manual quantitation using CellQuest and Microsoft® Excel software (refer to the QuantiBRITE PE data sheet).

**Internal Quality Control**

BD recommends using CaliBRITE™ beads and FACSComp™ software to set photomultiplier tube (PMT) voltages, fluorescence compensation, and to check instrument sensitivity prior to use. Refer to the CaliBRITE Beads package insert and the FACSComp Software User’s Guide.

BD recommends 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. PERFORMANCE CHARACTERISTICS**

**Specificity**

CD20 antigen is a phosphoprotein with a molecular weight of 35 or 37 kilodaltons (kd), depending on the degree of phosphorylation. The antigen is not glycosylated. CD20 antigen is expressed on B lymphocytes synchronous with the expression of surface IgM. The antigen is present on both resting and activated B lymphocytes but is lost before differentiation into plasma cells. The CD20 antigen is found in both mantle-zone and germinal-center areas of secondary follicles of lymphoid tissue and can be expressed on follicular dendritic cells (FDCs) in germinal centers. Low-level expression of the CD20 antigen has been detected on a subpopulation of T lymphocytes.

**Sensitivity**

Sensitivity is defined as the minimum number of ABC that can be reliably detected. The ABC that represent noise or non-specific staining for the CD20 antigen was based on monocytes, which...
are known to be negative for CD20. The minimum ABC was determined to be 745 (average plus three standard deviations).

**Reproducibility**

CD20 was submitted to the Third International Workshop and Conference on Human Leucocyte Differentiation Antigens. Participating laboratories evaluated clone L27 as part of a blind panel of antibodies and reported consistent results.²

**Repeatability**

To determine the repeatability of staining with each reagent, samples were stained with multiple lots of reagents. The different samples used in the evaluation provided an average mean fluorescence intensity (MFI) value as shown in Table 2. For each sample, two different lots of reagents generated a pair of results. Individual SDs were determined from the paired results for each sample. Individual SDs were combined to derive a pooled SD for each reagent that provides an estimate of within-sample repeatability.

**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.

As 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 data performance was collected typically with EDTA-treated blood. Reagent performance can be affected by the use of other anticoagulants.

| Table 2. Repeatability of mean fluorescence intensity (MFI) of B lymphocytes across different lots and across multiple donors (N) |
|---|---|---|---|
| PE | 8 | 3,698 | 224.1 | 6.06 |
| N = number of samples |
WARRANTY

The product sold hereunder is warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, that extend beyond the description on the label of the product. 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, 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 between debris and lymphocytes</td>
<td>Cell interaction with other cells and platelets</td>
<td>Prepare and stain another sample.</td>
</tr>
<tr>
<td>Rough handling of cell preparation</td>
<td>Inappropriate instrument settings</td>
<td>Check cell viability; centrifuge cells at lower speed. Follow proper instrument setup procedures; optimize instrument settings as required.</td>
</tr>
<tr>
<td>Staining dim or fading</td>
<td>Cell concentration too high at staining step</td>
<td>Check and adjust cell concentration or sample volume; stain with fresh sample. Repeat staining with increased amount of antibody.</td>
</tr>
<tr>
<td></td>
<td>Insufficient reagent</td>
<td>Repeat staining with fresh sample; analyze promptly. Use azide in staining medium and washing steps.</td>
</tr>
<tr>
<td></td>
<td>Cells not analyzed within 24 hours of staining</td>
<td>Use azide in staining medium and washing steps.</td>
</tr>
<tr>
<td></td>
<td>Improper medium preparation (azide omitted)</td>
<td>Use azide in staining medium and washing steps.</td>
</tr>
<tr>
<td>Few or no cells</td>
<td>Cell concentration too low</td>
<td>Resuspend fresh sample at a higher concentration; repeat staining and analysis. Troubleshoot instrument.</td>
</tr>
<tr>
<td></td>
<td>Cytometer malfunctioning</td>
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</table>

REFERENCES

