CaliBRITE Beads

CaliBRITE 3 three-color kit–Catalog No. 340486
CaliBRITE two-color kit–Catalog No. 349502
CaliBRITE PerCP Beads–Catalog No. 340497
CaliBRITE APC Beads–Catalog No. 340487
CaliBRITE PerCP-Cy5.5 Beads with Bead Dilution Buffer–Catalog No. 345036
For In Vitro Diagnostic Use with FACS brand flow cytometers

1/2011 23-3172-05

IVD

CE

1. INTENDED USE

BD CaliBRITE™ beads are designed for use with FACSComp™ or AutoCOMP™ software and the FACS™ family of flow cytometers (FACSCalibur™, FACSort™, FACScan™, and FACStrak™). The beads are used to adjust instrument settings, set fluorescence compensation, and check instrument sensitivity. Daily use is recommended for monitoring instrument performance over time. CaliBRITE beads are for in vitro diagnostic use.

2. SUMMARY AND EXPLANATION

CaliBRITE beads are available in two- and three-color kits. The two-color kit contains three different types of CaliBRITE beads: an unlabeled bead, a fluorescein isothiocyanate (FITC)-labeled bead, and a phycoerythrin (PE) -labeled bead. The three-color kit contains these beads plus a peridinin chlorophyll protein (PerCP) -labeled bead. An allophycocyanin (APC)-labeled bead is available separately and may be used with the three-color kit to perform four-color setup. CaliBRITE PerCP and PerCP -Cy5.5 beads are also available separately; PerCP-Cy5.5 beads can be used in the place of PerCP beads for applications using antibodies conjugated to the PerCP-Cy5.5 fluorochrome.

This package insert provides information for two-, three-, and four-color setup. Refer to the information appropriate to the instrument setup you are performing.

The flow cytometer has separate detectors or photomultiplier tubes (PMTs) that detect light signals. Both scatter and fluorescent light signals are detected. Because CaliBRITE beads simulate unstained cells and cells that have been stained (labeled) with fluorochrome-conjugated antibodies, the beads are used to adjust the instrument settings before cell samples are run on the flow cytometer.

The following list illustrates PMT light signal detection:

PMT	Primary Signal Detection
fluorescence-1 (FL1)	FITC (yellow-green)
fluorescence-2 (FL2)	PE (red-orange)
fluorescence-3 (FL3)	PerCP or PerCP-Cy5.5 (red)
fluorescence-4 (FL4)	APC (red)

Each fluorochrome emits light over a range of wavelengths when excited by the laser beam. Thus, a portion of the FITC signal is detected by the FL2 PMT, a portion of the PE signal is detected by the FL1 and FL3 PMTs; a portion of the PerCP or PerCP-Cy5.5 signal is detected by the FL4 PMT (PerCP and PerCP-Cy5.5 signals are not detected by the FL2 PMT); and a portion of the APC signal is detected by the FL3 PMT. This "spectral overlap" must be corrected using electronic compensation. CaliBRITE beads are used to determine the appropriate compensation settings.

After the instrument settings have been determined, CaliBRITE beads are used to evaluate instrument sensitivity. Forward scatter (FSC) and side scatter (SSC) instrument sensitivity are measured by the mean channel separation between the light-scatter signal of the beads and background signal (electronic and optical). FL1, FL2, and FL3 fluorescence sensitivity is determined by measuring the mean channel separation between the signal of the labeled beads and the unlabeled beads. FL4 sensitivity is determined by measuring separation between PerCP or PerCP-Cy5.5 and APC beads. A minimum channel separation must be met for the scatter and fluorescence parameters. This allows cells to be distinguished from sample debris or background signal and for dimly stained cells to be distinguished from unstained cells.

3. PRINCIPLES OF THE PROCEDURE

To prepare the flow cytometer for use, FACSComp (or AutoCOMP), using the CaliBRITE unlabeled bead suspension, sets an FSC gate to isolate singlet events from debris and multiple-bead events, then adjusts PMT voltages. The fluorescence PMT voltages are adjusted until the mean channel values for the unlabeled beads correspond to predetermined target values (for the FL4 PMT, FACSComp sets the mean channel value for the APC bead to a predetermined target value). The SSC PMT voltage is adjusted to position the beads at their SSC target channel. The FSC threshold is adjusted to a level that minimizes background signal (if any).

Next, the software adjusts fluorescence compensation using a mixed-bead suspension containing equal amounts of the appropriate CaliBRITE beads. For two-color instrument setup, the fluorescence compensation adjustments are FL1–%FL2 and FL2–%FL1. For three-color setup, the fluorescence compensation adjustment is FL3–%FL2. (Because PerCP and PerCP-Cy5.5 are not detected by the FL2 PMT, FL2–%FL3 compensation is not necessary.) For four-color setup, the fluorescence compensation adjustments are FL3–%FL4 and FL4–%FL3.

Compensation adjustments for FL1, FL2, and FL3 correct for spectral overlap by shifting the labeled bead populations so they are aligned with the corresponding unlabeled bead populations. FL4 compensation is set by placing APC beads in a specified target channel along the FL3 axis and PerCP or PerCP-Cy5.5 beads in a specified target channel along the FL4 axis. Refer to the *FACSComp Software User's Guide* for details.

Following PMT and compensation adjustment, the software performs a Sensitivity Test using the appropriate mixed-bead suspension.

NOTE: Because leucocytes have different optical properties than CaliBRITE beads, normal donor peripheral blood samples are recommended for optimizing FSC and SSC gains, FSC threshold, and fluorescence compensation levels. See Optimization and Quality Control on page 3. Figures 1–4 show examples of optimization for two-, three-, and four-color applications.

4. REAGENTS

- CaliBRITE beads contain individual vials of polymethylmethacrylate microspheres of approximately 6 µm: 2.5 mL of unlabeled beads, 1.25 mL of FITC-labeled beads, 1.25 mL of PE-labeled beads, 1.25 mL of PerCP-labeled beads, 1.25 mL of PerCP-Cy5.5–labeled beads, and 2.5 mL of APC-labeled beads. All forms are provided in stabilized, buffered saline with 0.1% sodium azide (see Precautions). Reagents are sufficient to perform 25 tests.

- Bead Dilution Buffer contains 100 mL of stabilized buffer with 0.1% sodium azide (see Precautions). One bottle is sufficient to perform 25 tests.

Storage and Handling

- Store CaliBRITE beads at 2° to 8°C and protected from direct light. Do not use after the expiration date shown on the label.
- Store Bead Dilution Buffer at 2° to 8°C.
- After dilution, CaliBRITE bead suspensions prepared in Bead Dilution Buffer are stable for 8 hours at 2° to 8°C or for 1 hour at 20–25°C.
- After dilution, CaliBRITE bead suspensions prepared in FACSFlow™ sheath fluid are stable for 8 hours at 2° to 8°C or, if PerCP beads are included, for 1 hour at 2° to 8°C.

Precautions

1. For in vitro diagnostic use.
2. Do not freeze CaliBRITE beads or expose them to direct light during storage or use.
3. PerCP-Cy5.5 beads substitute for PerCP beads; do not put both together into the same tube.
4. **CAUTION:** Dilute CaliBRITE beads only in FACSFlow sheath fluid (BD Catalog No. 342003) or Bead Dilution Buffer (BD Catalog No. 345035) as directed in Section 6, Procedure. Do not dilute PerCP-Cy5.5 beads in sheath fluid.
5. Do not use CaliBRITE beads beyond their expiration date or beyond the stability period described in Storage and Handling. Beads used beyond their stability begin to show a decrease in separation between unlabeled and labeled populations, possibly resulting in Sensitivity Test failure.
6. Bead aging can decrease fluorescence separation by as much as 2.5 channels per month.
7. Excessive changes in results can indicate deterioration of the beads or changes in instrument conditions. If deterioration is suspected, prepare a new bead suspension and check instrument conditions.
8. For optimization of the flow cytometer before running the samples, see Optimization and Quality Control on page 3.
9. The reagents contain sodium azide as a preservative; however, use care to avoid microbial contamination, which can cause erroneous results.

   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.

5. INSTRUMENT

FACSCalibur, FACSort, FACScan, or FACStrak

CaliBRITE beads are intended for use on a BD FACS brand flow cytometer equipped for two-, three-, and four-color fluorescence detection and two-parameter light-scatter detection. (FACStrak is equipped for two-color fluorescence detection only. The FACSCalibur and FACSort, equipped with the FL4 option, are capable of four-color fluorescence detection.) For information on use, refer to the appropriate instrument manual.

The cytometer must be equipped with FACSComp or AutoCOMP software, version 2.0 or greater. For detailed information on use, refer to the appropriate software user's guide.

NOTE: FACSComp software version 4.2 or greater is required for use with PerCP-Cy5.5 beads.

6. PROCEDURE

Reagent Provided

See Precautions in Section 4, Reagents.

Reagents and Materials Required but Not Provided

- Disposable 12 x 75-mm Falcon™ capped polystyrene test tubes (BD Catalog No. 352058) or equivalent.
- Micropipettor with tips (BD Electronic Pipette, BD Catalog No. 646539) or equivalent.
- FACSFlow sheath fluid (BD Catalog No. 342003).
- Samples stained with monoclonal antibodies that identify separate, non-overlapping cell populations might be necessary for optimizing instrument settings. See examples in Optimization and Quality Control on page 3.

Preparation of Test Suspensions

Prepare all bead suspensions immediately prior to use. Mix bead vials by gentle inversion or very gentle vortexing prior to use.

1. Label two 12 x 75-mm polystyrene tubes Tube A and Tube B.
2. Dispense 1 mL of sheath fluid or Bead Dilution Buffer into Tube A.
3. Dispense 3 mL of sheath fluid or Bead Dilution Buffer into Tube B.

   CAUTION: Use only Bead Dilution Buffer for calibrations with PerCP-Cy5.5 beads; do not use sheath fluid.
4. Gently mix the CaliBRITE bead vials, then add 1 drop of beads to each tube as indicated in the table below.

   NOTE: Invert bead vials completely when adding a drop to the tube. Make sure to obtain a full drop of beads. The drop should be cloudy, indicating the beads are properly mixed.

Setup	Tube [a]	Unlabeled	FITC	PE	PerCP or PerCP-Cy5.5 [b]	APC
two-color	A	1 drop				
	B	1 drop	1 drop	1 drop		
three-color	A	1 drop				
	B	1 drop	1 drop	1 drop	1 drop	
four-color	A	1 drop				1 drop
	B	1 drop	1 drop	1 drop	1 drop	1 drop

a. Use Tube A for PMT adjustment; use Tube B for fluorescence compensation and sensitivity testing.
b. PerCP-Cy5.5 beads substitute for PerCP beads; do not put both together into the same tube.

5. Keep prepared bead suspensions on ice or at 2° to 8°C and protect from direct light at all times.
   - CaliBRITE bead suspensions prepared in Bead Dilution Buffer are stable for 8 hours at 2° to 8°C or for 1 hour at 20° to 25°C.

- CaliBRITE bead suspensions prepared in FACSFlow sheath fluid are stable for 8 hours at 2° to 8°C or, if PerCP beads are included, for 1 hour at 2° to 8°C. Do not dilute PerCP-Cy5.5 beads in sheath fluid.

System Setup

For detailed information on using FACSComp or AutoCOMP software with CaliBRITE beads for instrument setup, refer to the *FACSComp Software User's Guide* or the *AutoCOMP Software Reference Manual*.

1. Adjust PMT voltage settings using Tube A.
2. Adjust fluorescence compensation using Tube B.
3. Perform a Sensitivity Test using Tube B.
4. Generate a printout of the Sensitivity Test results and keep the printouts in a log book. Record PMT voltages and channel separations obtained for each parameter in a daily log sheet.
5. Optimize settings for your sample, as needed.

   Instrument settings might need to be manually optimized before running cells. Visually inspect dot plots for proper PMT gains, compensation, and FSC threshold (see Figures 1–5 in the following section, Optimization and Quality Control).

Optimization and Quality Control

Because leucocytes have different optical properties than CaliBRITE beads, optimization of instrument settings with cell samples is important. Prepare a blood sample daily from a normal donor. Use the same staining method and run in parallel with the test samples. Optimize instrument settings following two-color setup using a blood sample stained with any combination of monoclonal antibodies that identifies separate non-overlapping cell populations such as FITC-labeled and PE-labeled monoclonal antibodies. Optimization following three- and four-color setup can vary depending on the application. See Figures 1–5 for examples. Always refer to the appropriate application note or reagent package insert.

NOTE: Different immunophenotyping preparation methods might require different optimization procedures. It might be necessary to adjust the FSC and SSC amplifiers so that all leucocyte populations are on scale, and to adjust compensation and threshold settings (see Figure 1).

Optimizing Scatter

Figure 1 shows a lysed whole blood (LWB) sample from a normal donor before and after optimization. Notice populations with a lower FSC signal than lymphocytes (debris, for example) can be excluded by increasing the FSC threshold level.

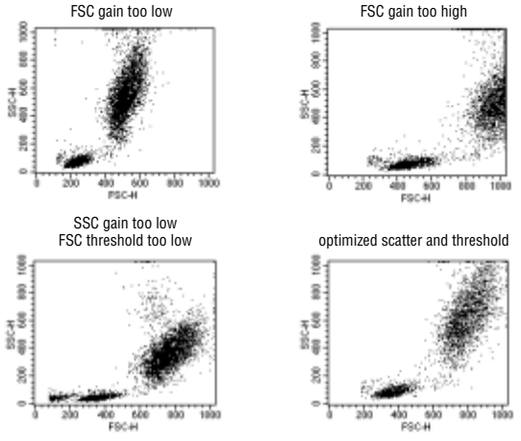

Figure 1 FSC vs SSC dot plots before and after optimization

Optimizing Compensation

Figure 2 shows a normal peripheral LWB sample before and after FL1 vs FL2 compensation adjustment. The FL2–%FL1 compensation level is adjusted so the FITC (FL1) population is aligned along the y-axis with the unlabeled bead population. The FL1–%FL2 compensation level is adjusted so the PE (FL2) population is aligned along the x-axis with the unlabeled bead population.

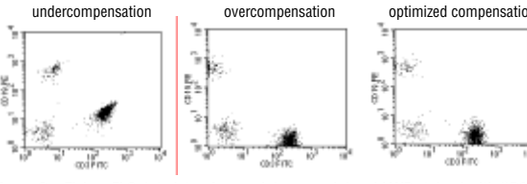

Figure 2 FL1 vs FL2 dot plots of normal donor peripheral LWB stained with CD3 FITC/CD19 PE

Figure 3 shows a normal peripheral blood sample before and after FL3 vs FL2 compensation adjustment. The FL3–%FL2 compensation level is adjusted so the PE (FL2) population is aligned along the x-axis with the negative population. FL2–%FL3 compensation is not necessary.

Figure 3 FL3 vs FL2 dot plots of normal donor peripheral LWB stained with IgG_1 FITC/CD8 PE/IgG_1 PerCP using a lyse/wash method

Figures 4 and 5 show a normal peripheral blood sample before and after FL3 vs FL4 compensation adjustment. In Figure 4, the FL4–%FL3 compensation level is adjusted so the PerCP-stained population is moved from the center of the plot to the x-axis. Adjustment is similar for PerCP-Cy5.5–stained samples. The required FL4–%FL3 compensation level is usually less than that for PerCP.

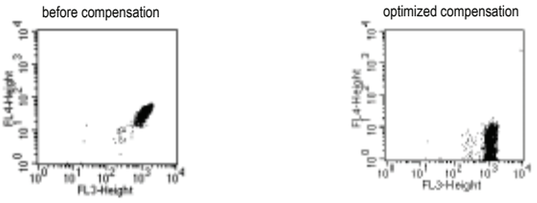

Figure 4 FL3 vs FL4 dot plots of normal donor peripheral LWB stained with IgG_1 FITC/ IgG_1 PE/ CD45 PerCP/IgG_1 APC

In Figure 5, the FL3–%FL4 compensation level is adjusted so the APC-stained population is aligned along the x-axis with the negative population.

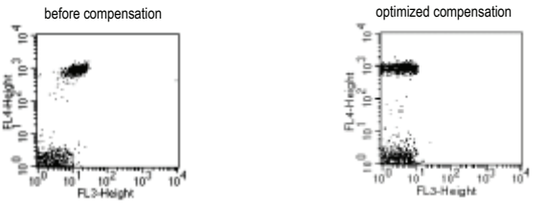

Figure 5 FL3 vs FL4 dot-plot displays of normal donor peripheral LWB stained with IgG_1 FITC/ IgG_1 PE/ IgG_1 PerCP/CD4 APC

7. RESULTS

When using CaliBRITE beads, the fluorescence sensitivity is determined by the amount of channel separation between the unlabeled and labeled bead populations. For FL4, the fluorescence sensitivity is determined by the amount of channel separation between APC and PerCP beads. The light scatter sensitivity is determined by the amount of channel separation between the mixed bead population and instrument background signal. The channel separation and PMT voltages for each of the four parameters should be maintained in a daily log to track instrument performance.

NOTE: Over a period of time, the fluorescence separation might decrease. The decrease in separation for a wide variety of bead lots has been within 2.5 channels per month. Corrective action might be required if the average separation varies by more than 2.5 channels per month (see Section 9, Troubleshooting).

For the same lot of beads observed over 26 days on a single instrument at a clinical site, PMT settings varied as much as 13 volts standard deviation (SD) for FL1, 12 volts SD for FL2, 12 volts SD for FL3, and 10 volts SD for FL4. On a single instrument at BD Biosciences (Immunocytometry Systems), PMT settings over various lots of beads varied by 7 volts SD for FL1, 8 volts SD for FL2, 9 volts SD for FL3, and 10 volts SD for FL4. Observations of greater variations on a single instrument can be indicative of instrument instability.

8. LIMITATIONS

CaliBRITE beads are recommended for use with FACSComp or AutoCOMP software on a BD FACSCalibur, FACSort, FACScan, or FACStrak flow cytometer.

In some cases the software may not be able to automatically set up the instrument. If this occurs, manually adjust the settings. Refer to the *FACSComp Software User's Guide*.

9. TROUBLESHOOTING

If the CaliBRITE beads do not meet the required minimum sensitivity specifications for the flow cytometer used, check the following:

- Make sure the beads have not passed the expiration date printed on the label.
- If the prepared suspension is not fresh, make up a fresh bead suspension and repeat the procedure. Beads used beyond their stability begin to show a decrease in separation between unlabeled and labeled populations, resulting in Sensitivity Test failure.
- Use Bead Dilution Buffer rather than sheath fluid to prepare bead suspensions. The suspensions are stable for a longer period of time in Bead Dilution Buffer.
- Use new sheath fluid or Bead Dilution Buffer to dilute beads if you suspect microbial contamination.
- Open new bead vials if you suspect the beads are contaminated.
- Check instrument fluidics for bubbles or debris. If instrument cleaning is necessary, refer to the flow cytometer user's guide for instructions.

For additional troubleshooting information, refer to the *FACSComp Software User's Guide* or the *AutoCOMP Software Reference Manual*.

For further assistance, contact your BD Biosciences service representative.

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.

CUSTOMER SUPPORT INFORMATION

Becton, Dickinson and Company
BD Biosciences
San Jose, CA 95131
Tel 877.232.8995
Fax 408.954.2347
ClinicalApplications@bd.com

BD Biosciences
European Customer Support
Tel 32.2.400.9895
Fax 32.2.401.7094
help.biosciences@europe.bd.com

bdbiosciences.com

EC REP **BENEX Limited**
Rineanna House
Shannon Free Zone
Shannon, County Clare
Ireland
Tel 353.61.472920
Fax 353.61.472907