



## BD™ CS&T Beads

Catalog No.	Tests
656504	50
656505	150

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## 1. INTENDED USE

BD™ CS&T beads are used on a BD flow cytometer to provide a standardized method to perform quality control of the instrument's optics, electronics, and fluidics, and for adjusting fluorescence compensation. On some BD instruments, BD CS&T beads are also used for adjusting detector voltages.

## 2. SUMMARY AND EXPLANATION

BD CS&T beads are a suspension of fluorospheres with uniform and stable size and fluorescence intensity. The beads are used for instrument quality control (QC) to characterize, track, and report performance measurements of supported flow cytometers. The cytometer's software displays current bead data in plots. Forward scatter (FSC) and side scatter (SSC) identify bead populations based on relative size.

The beads enable the software to measure detector performance and are used to measure the sensitivity of each fluorescence detector. Sensitivity is a measure of the cytometer's ability to resolve dimly stained cells. In addition, the beads are used to optimize the compensation settings each time instrument QC is run.

The software calculates the bright bead median, bright beads %rCV (robust coefficient of variation), and instrument sensitivity for FSC, SSC, and each fluorescence parameter, and compares them to expected values for the bead lot. The rCV measures cytometer alignment.

Daily measurements are automatically entered into Levey-Jennings plots. This allows you to monitor instrument

performance measurements over time and detect potential problems.

For the BD FACSLytic™ flow cytometer, BD CS&T beads are also used for adjusting detector voltages.

### 3. PRINCIPLES OF THE PROCEDURE

BD CS&T beads consist of equal quantities of 3-µm bright, 3-µm mid, and 2-µm dim polystyrene beads. The beads are dyed with fluorochromes. Fluorescence intensity is measured by the cytometer's detectors, processed by the electronics, and displayed and analyzed by the software.

The cytometer's software reads the bead lot file and calculates the target marker position or target fluorescence intensity for the beads. It then calculates the median and %rCV for bright beads.

### 4. REAGENTS

#### Reagents provided

BD CS&T beads are supplied in phosphate buffered saline (PBS) with bovine serum albumin (BSA) and 0.1% sodium azide as follows.

- Two 3-mL vials (50 tests)
- Six 3-mL vials (150 tests)

Each 3-mL vial contains sufficient beads to run 25 tests.

#### Reagents or materials required but not provided

- Disposable 12 × 75-mm capped polystyrene test tubes
- Filtered deionized (DI) water, to dilute the beads (BD FACSVia™ system only)
- BD FACSTream™ sheath fluid (Catalog No. 342003) or equivalent, to dilute the

beads (BD FACSLytic flow cytometer only)

### Precautions

- Avoid exposing BD CS&T beads to direct light.
- Do not run BD CS&T beads without first diluting them with the proper diluent, as directed in the Procedure section.
- Do not use BD CS&T beads beyond their expiration date or beyond the day-of-use stability period after dilution. Beads used beyond their stability period begin to lose fluorescence, which can result in failed instrument QC.
- BD CS&T beads contain sodium azide as a preservative.

### Storage and handling

- Store vials at 2°C–8°C and protect from light. Do not use after the expiration date shown on the label.

### 5. INSTRUMENTS

BD CS&T beads are for use on the following:

- BD FACSVia flow cytometer
- BD FACSLytic flow cytometer

### 6. PROCEDURE

#### Adding or importing bead lot information

Add bead lot ID information by scanning the bead lot file card in this kit.

If you do not have a barcode scanner, import bead lot ID information from the BD Biosciences website.

1. Visit [bdbiosciences.com](http://bdbiosciences.com) and select **Support** from the menu bar.

The **Services** web page opens.

- From **Top Support Links** in the right panel, select **Bead Lot Files** for the appropriate software.
- Follow the installation instructions on the website to download and import the appropriate bead lot file into the software.

### Preparing a BD CS&T bead suspension

Carefully read the Precautions and Storage and handling statements in the Reagents section.

To prepare the BD CS&T beads for acquisition:

- Label a 12 × 75-mm capped polystyrene tube.
- Thoroughly mix the BD CS&T beads vial. Invert the vial 10 times, or vortex the vial at medium speed for 5–10 seconds.
- Prepare diluted beads according to Table 1 for the system and application you are running.

**NOTE** Avoid dripping the beads down the side of the tube when diluting them. This can lead to low bead counts during acquisition.

**NOTE** Do not dilute BD CS&T beads more than recommended.

- Vortex the tubes gently before use.

After dilution, the beads are stable for:

- 8 hours at 2°C–25°C on the BD FACSVia system
- 8 hours at 15°C–25°C, or 24 hours at 2°C–8°C on the BD FACSLytic flow cytometer.

**NOTE** Keep the diluted bead suspension protected from light. Some of the dyes used to manufacture the beads are light sensitive. Fluorescence levels can change if beads are exposed to direct light for longer than 20 minutes.

**Table 1** BD CS&T beads preparation

For...		Add...				
Task	Cytometer	Diluent	Diluent volume (µL)	Beads (No. of drops)	To tube labeled	How often
Instrument QC (IQC)	BD FACSVia	Filtered DI water	500	2	IQC	<ul style="list-style-type: none"> <li>• Daily</li> <li>• When recommended by BD</li> <li>• To transfer bead lots</li> </ul>

**Table 1** BD CS&T beads preparation

For...		Add...				
Task	Cytometer	Diluent	Diluent volume (µL)	Beads (No. of drops)	To tube labeled	How often
Performance QC <sup>a</sup> (PQC)	BD FACSLyric	BD FACSThroughput sheath fluid	500	2	PQC	Daily
Update reference settings			500	2	Ref	Every 60 days
Bead lot transfer			500	2	Old lot	Before using a new lot
			500	2	New lot	
Characterization QC (CQC)			1,000	4	CQC	<ul style="list-style-type: none"><li>• Every 6 months</li><li>• After service or maintenance</li><li>• When recommended by BD</li></ul>
Laser setup			1,000	4	Laser	As necessary

a. Assay and tube settings are automatically updated when running performance QC.

## Performing QC on the instrument using BD CS&T beads

Run instrument QC according to Table 1. See the instrument's Instructions For Use (IFU) for instructions on installing a bead lot file and performing any of the tasks outlined in Table 1.

## 7. RESULTS

### Reviewing the Instrument QC Report

The Instrument QC Report contains the cytometer serial number, software version, BD CS&T bead lot information, bright bead median, bright beads %rCV, instrument sensitivity, and a pass or fail result for each parameter. A passing result for every parameter is required for instrument QC to pass. A failure for any parameter results in failure of instrument QC. For troubleshooting any QC messages, see the cytometer's IFU.

When using the BD FACSVia system, we recommend that you visually review the marker positions for scatter and bright

bead peaks and adjust the markers to surround the bead population, as necessary.

## 8. LIMITATIONS

- BD CS&T beads are intended for use with supported flow cytometers and their applicable software.
- BD CS&T beads should not be used to support quantitative fluorescence measurements in a flow cytometer.
- BD CS&T beads are for instrument QC and setup only.

## 9. PERFORMANCE CHARACTERISTICS

Performance of the BD CS&T beads was established by testing at BD Biosciences laboratories in San Jose, CA, USA.

### Accuracy

Assay settings and tube settings were determined three times on one BD FACSLyric flow cytometer using one lot of BD CS&T beads. For each

fluorescence channel, the bright bead median fluorescence intensity (MFI) value (Actual), generated from the assay setup reports, was compared with the lot-specific bright bead MFI value (Target).

Accuracy was calculated as the percent difference between the bright bead MFI values of the Actual and the bright bead MFI values of the Target. See Table 2.

**Table 2** Accuracy of cytometer setup using BD CS&T beads<sup>a</sup>

Parameter	Bright bead MFI		% Difference
	Target	Actual	
FSC	17,991	17,992	0.006
SSC	126,269	126,459	0.150
FITC	5,952	5,930	-0.370
PE	12,719	12,700	-0.149
PerCP-Cy5.5	17,875	17,950	0.420
PE-Cy7	16,237	16,250	0.080
APC	40,693	40,901	0.511
APC-R700 <sup>b</sup>	42,873	42,951	0.182
APC-Cy7	85,174	85,397	0.262
V450 <sup>a</sup>	6,203	6,219	0.258
V500-C <sup>a</sup>	24,488	24,483	-0.020
BV605 <sup>a</sup>	6,423	6,393	-0.467

a. The data presented are from one run with LNW tube settings. Results from LW tube settings and the other runs were similar.

b. BD Horizon™ APC-R700, BD Horizon™ V450, BD Horizon™ V500-C, BD Horizon Brilliant™ Violet 605

## Reproducibility

Instrument CQC was run on two BD FACSLyric flow cytometers. Two operators performed two runs of instrument PQC in duplicate on each instrument every day for a period of eight days using one lot of BD CS&T beads. Percent coefficient of variation (%CV) of

the median MFI values for each channel in high sensitivity and normal modes was used to verify reproducibility. See Table 3.

**Table 3** Reproducibility of BD CS&T beads (Operator/instrument-to-operator/instrument, day-to-day, tube-to-tube)<sup>a</sup>

Parameter	%CV (High sensitivity)	%CV (Normal)
FSC	0.98	0.94
SSC	0.73	0.61
FITC	0.46	0.22
PE	0.41	0.24
PerCP-Cy5.5	0.88	0.78
PE-Cy7	1.28	1.23
APC	0.77	0.80
APC-R700	0.60	0.61
APC-Cy7	0.67	0.71
V450	0.67	0.65
V500-C	0.53	0.52
BV605	0.54	0.48

a. The data presented are from one instrument. Results from the other instrument were similar.

## Repeatability

Instrument CQC and PQC were each performed ten times on two BD FACSLyric flow cytometers using two lots of BD CS&T beads to assess run-to-run repeatability. The %CV of the bright beads %rCV (resolution), Br (background), minimum linearity, maximum linearity, and SDen (standard deviation of the electronic noise) were used to verify run-to-run repeatability. See Table 4.

**Table 4** Run-to-run repeatability of instrument CQC using BD CS&T beads<sup>a</sup>

Parameter	Bright bead rCV		SD for Br <100	%CV for Br ≥100	SD for linearity minimum (<500)	%CV for linearity maximum	%CV for SDen
	%CV of rCV	SD of rCV <2%					
FSC	NA	0.07	NA	NA	NA	NA	NA
SSC	NA	0.03	NA	NA	NA	NA	NA
FITC	NA	0.05	NA	5.01	24.04	0.14	2.87
PE	NA	0.06	NA	4.97	23.84	0.15	3.09
PerCP-Cy5.5	2.36	NA	2.67	NA	16.05	0.15	2.43
PE-Cy7	0.88	NA	0	NA	11.55	0.20	1.86
APC	NA	0.07	1.42	NA	22.41	0.32	1.64
APC-R700	NA	0.06	4.10	NA	29.58	0.26	2.14
APC-Cy7	NA	0.09	26.64	NA	22.23	0.34	1.63
V450	0.96	NA	NA	7.0	23.20	0.25	2.82
V500-C	1.58	NA	NA	6.34	46.83	0.32	2.25
BV605	1.44	NA	2.67	NA	47.54	0.26	1.45

a. The data presented are for one lot of BD CS&T beads run on one instrument. Results for the remaining lots of beads and instruments were similar.

## TROUBLESHOOTING

Problem	Possible Causes	Solution
No beads detected	Beads not mixed prior to diluting, beads are too dilute, there is debris in the bead suspension, incorrect beads were used, beads diluted in wrong diluent, beads exposed to light	<ol style="list-style-type: none"> <li>1. Vortex the bead vial.</li> <li>2. Prepare a fresh suspension of beads.</li> <li>3. Re-run instrument QC.</li> </ol>
	Air bubbles in the flow cell or sheath filter	<ol style="list-style-type: none"> <li>1. For: <ul style="list-style-type: none"> <li>• BD FACSVia, perform a backflush or SIP clean.</li> <li>• BD FACSLytic, perform a SIT flush.</li> </ul> </li> <li>2. Vortex the tube.</li> <li>3. Re-run the tube.</li> </ol>
	Sheath filter is not filled with fluid	<ul style="list-style-type: none"> <li>• For BD FACSVia, perform the two-month maintenance procedure.</li> <li>• For BD FACSLytic, purge the sheath filter.</li> </ul>
No beads detected	Clogs within the sample path and fluidic lines	<ol style="list-style-type: none"> <li>1. For: <ul style="list-style-type: none"> <li>• BD FACSVia, perform a backflush or SIP clean.</li> <li>• BD FACSLytic, perform a SIT flush.</li> </ul> </li> <li>2. Vortex the tube.</li> <li>3. Re-run the tube.</li> </ol>
	Optics are out of alignment	<ul style="list-style-type: none"> <li>• Contact BD Biosciences.</li> </ul>

Problem	Possible Causes	Solution
Performance check completed with QC messages	Bead gates and markers are not properly adjusted to encapsulate results	For BD FACSVia, review instrument QC results and adjust the CS&T Bead gates for scatter and fluorescence.
	Values for any measurements used to check cytometer performance are not within parameters required for instrument QC to pass (see Reagents section)	Prepare a fresh suspension of beads and re-run instrument QC.  <ol style="list-style-type: none"> <li>1. For: <ul style="list-style-type: none"> <li>• BD FACSVia, perform the two-month maintenance procedure.</li> <li>• BD FACSLytic, perform the monthly cleaning procedure.</li> </ul> </li> <li>2. Re-run the tube.</li> </ol>
		Review the instrument QC report to determine whether the specific warnings impact the assay, then continue.
		Contact BD Biosciences.
Performance check failure	Value(s) for any of the measurements used to check the cytometer performance are not within parameters required for instrument QC to pass (see Section 7)	<ol style="list-style-type: none"> <li>1. Prepare a fresh suspension of beads.</li> <li>2. Re-run the performance check.</li> </ol> Perform the monthly cleaning procedure.
	Improper ratio of 2 $\mu\text{m}$ /3 $\mu\text{m}$ beads due to inadequate mixing of beads	<ol style="list-style-type: none"> <li>1. Prepare a fresh suspension of beads.</li> <li>2. Re-run the performance check.</li> <li>3. If QC fails again, prepare beads from a new vial and be sure to thoroughly vortex the vial prior to use. See Preparing a BD CS&amp;T bead suspension.</li> </ol>

For additional troubleshooting assistance, see the cytometer's IFU or contact your local BD Biosciences representative.

## **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.

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