BD™ FC Beads 8-Color Kit for BD OneFlow™ Assays

5 tests per kit—Catalog No. 658621

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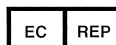

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1. INTENDED USE

BD™ FC beads 8-color kit for BD OneFlow™ assays (BD FC beads) are intended to allow software to determine spillover values (SOVs) for fluorescence compensation. BD FC beads are designed for use with a suitably equipped BD™ flow cytometer and software designated for in vitro diagnostic use.

2. SUMMARY AND EXPLANATION

BD FC beads are fluorescent beads that enable BD FACSDiva™ software to calculate a fluorescence compensation matrix during setup of the BD FACSCanto™ II flow cytometer.

BD FC beads are 3-µm polystyrene beads coupled to fluorochromes and dried in single-use 12 x 75-mm tubes that are rehydrated with bead dilution buffer immediately before use.

3. PRINCIPLES OF THE PROCEDURE

BD has developed a suite of beads that are used with BD FACSDiva software to standardize setup of the BD FACSCanto II flow cytometer with a 3-laser, 8-color 4-2H-2V BD default (4-2H-2V) optical configuration. First, BD FACSDiva™ CS&T IVD beads (CS&T IVD beads) are used to perform daily cytometer quality control. BD OneFlow™ Setup beads (BD OneFlow Setup beads) and lysed washed blood (LWB) are then used to set assay-specific detector photomultiplier tube voltages (PMTVs) and to generate Application Settings. Finally, BD FC beads are used to calculate compensation.

4. STORAGE AND HANDLING

- Store tubes at 2°C–8°C in the foil pouch. The tubes should not be frozen. Protect the tubes from exposure to light and humidity. The beads and diluent are stable until the expiration date shown on the pouch and bottle labels when stored as directed. Do not use after the expiration date.

  CAUTION Do not remove the desiccant pack from the pouch. Reseal the pouch immediately after removing a tube and return the pouch to 2°C–8°C storage as soon as possible.
- After rehydration, when protected from light, the beads are stable for:
  - 1 hour at 25°C
  - 4 hours at 2°C–8°C

5. REAGENTS AND MATERIALS

Reagents and Materials Provided

- BD FC beads

  Includes one pouch of 5 tubes for each of the following fluorochromes:
  - FITC
  - PE
  - PerCP-Cy™* 5.5
  - PE-Cy™7
  - APC
  - APC-H7
  - BD Horizon™ V450
  - BD Horizon™ V500-C
- BD™ FC beads dilution buffer

  The BD FC beads dilution buffer contains phosphate buffered saline (PBS) with protein stabilizers and 0.1% sodium azide.

Reagents and Materials Required but Not Provided

- Vortex mixer
- BD FACSCanto II flow cytometer with a 4-2H-2V optical configuration

  See the cytometer user's guide for information.
- BD FACSDiva software v8.0.1 or later

  See the *BD FACSDiva Software Reference Manual*.
- BD FACSDiva CS&T IVD beads (CS&T IVD beads) (Catalog No. 656046 or 656047)

  See the *BD FACSDiva CS&T IVD Beads* IFU.
- BD OneFlow™ BD Setup Beads (Catalog No. 658620)

  See the *BD OneFlow™ BD Setup Beads* IFU.

Precautions

- Do not use BD FC beads tubes beyond their expiration date or beyond the day-of-use stability period after rehydration, as described in the Storage and Handling section.

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6. PROCEDURE

Generate new SOVs using BD FC beads at the following times:

- At least once a month
- Each time new Application Settings are generated
- Each time a new BD OneFlow Setup beads lot is used to generate Application Settings
- Whenever a new baseline is defined using CS&T IVD beads
- After cytometer maintenance or service is performed

Preparing BD FC beads

CAUTION Before preparing BD FC beads, verify that the:

- CS&T IVD beads daily performance check for the 4-2H-2V configuration was completed today and passed
- Detector voltages have been adjusted and Application Settings have been generated according to the *BD OneFlow™ BD Setup Beads* IFU

1. Allow the bead pouches to reach 18°C–25°C before opening each pouch.
2. Open a pouch, remove one tube, and place it in a rack protected from light.

   CAUTION Do not remove the desiccant pack from the pouch.

3. Re-seal the pouch immediately, write the date it was first opened on the pouch label, and return it to 2°C–8°C storage as soon as possible.

   WARNING Protect the bead tubes from light before and after reconstitution. Some of the dyes used to manufacture the beads are very light sensitive. Fluorescence SOVs can change if the beads are exposed to light.

4. Repeat steps 2 and 3 for the remaining fluorochrome tubes.
5. Add 0.5 mL of BD FC beads dilution buffer to each tube.

   CAUTION Use only the BD FC beads dilution buffer included with the kit. Use of other diluent could result in incorrect SOVs.

6. Vortex the tubes vigorously for 3–5 seconds.

   If not acquiring immediately, store the rehydrated bead tubes at 2°C–8°C protected from light. After rehydration, the beads are stable for:

   - 1 hour at 18°C–25°C
   - 4 hours at 2°C–8°C

Creating compensation controls

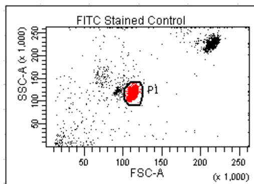
1. In the title bar of the BD FACSDiva workspace, confirm that the 4-2H-2V optical configuration is selected.
2. From the menu bar, select **Experiment > New Experiment > Blank Experiment > OK**.
3. If prompted by the CST Mismatch dialog, select **Use CST Settings**.
4. In the Browser, right-click the **Cytometer Settings**, and select **Application Settings > Apply**.
5. In the Application Settings dialog, select the Applications Settings previously created using BD Setup Beads and LWB and click **OK.**

6. In the Cytometer Settings Mismatch dialog, click **Overwrite.**
7. From the menu bar, select **Experiment > Compensation Setup > Create Compensation Controls.**

   The Create Compensation Controls dialog opens.
8. Clear the **Include separate unstained control tube/well** checkbox.
9. Select generic (default) labels for FITC, PE, PerCP-Cy5.5, PE-Cy7, APC, APC-H7, V450, and V500.
10. Click **OK.**

Acquiring compensation controls

1. Vortex the FITC stained control tube.
2. Install the tube on the cytometer.
3. In the **Browser,** select the current tube pointer for the FITC stained control tube.
4. In the Acquisition dashboard:
   - Confirm that **Events to Record** is set to 5,000 total events
   - Adjust the flow rate to **Medium**
5. Click **Acquire Data.**
6. Click the **P1** gate in the FSC-A vs SSC-A dot plot and adjust the gate to fully encompass the singlet bead population.

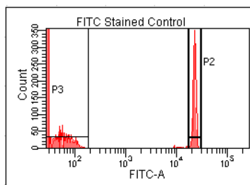


NOTE Increase the FSC PMTV to resolve the singlet bead population.

7. Right-click the **P1** gate border and select **Apply to all compensation controls.**
8. Click **Record Data** to record 5,000 events for the BD FC beads FITC control tube.
9. Verify that the **P2** interval gate encompasses the FITC-positive population. See Figure 1.
10. Add a **P3** interval gate to the histogram and ensure that it encompasses the negative population.

    NOTE Events can stack up on the y-axis. Verify that the left side of the **P3** interval gate starts at the y-axis.

Figure 1 FITC control showing P2 and P3

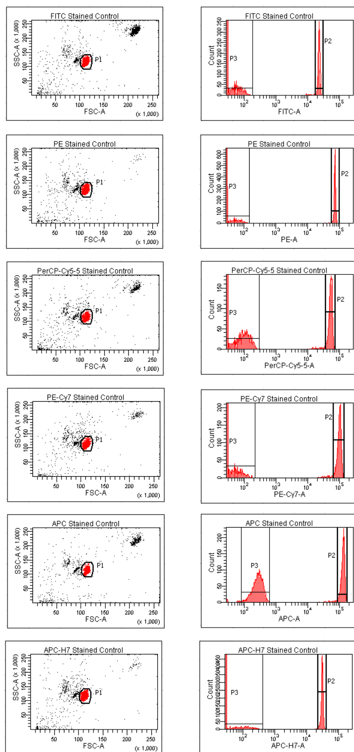


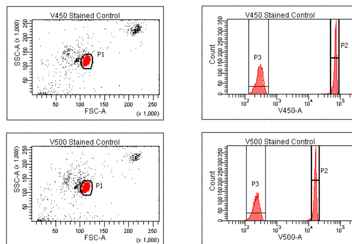
11. Select the next tube.
12. Repeat steps 1 through 11 for the remaining fluorochrome tubes. See Figure 2 for representative data.
13. From the menu bar, select **Experiment > Compensation Setup > Calculate Compensation.**
14. Name the compensation file with the run date appended with OneFlow (for example, OneFlow FC beads_today's date).
15. Select **Link and Save.**

Flow Cytometry

Figure 2 displays representative BD FC beads data acquired and analyzed on a BD FACSCanto II flow cytometer with a 4-2H-2V optical configuration and BD FACSDiva software. Laser excitation is at 488 nm (FITC, PE, PerCP-Cy5.5, PE-Cy7), 640 nm (APC, APC-H7), and 405 nm (V450, V500-C). Detector voltages were generated using BD Setup Beads.

Figure 2 Representative data





7. LIMITATIONS

- BD FC beads are intended to enable software to determine SOVs for fluorescence compensation.
  BD FC beads are designed for use with a BD FACSCanto II flow cytometer with a 4-2H-2V optical configuration and BD FACSDiva software v8.0.1 or later.
- BD FC beads do not perform as a fluorescence calibrator and should not be used for setting up a flow cytometer for quantitative fluorescence measurements.

8. PERFORMANCE CHARACTERISTICS

Accuracy

Multiple operators performed one run per day over multiple days on multiple BD FACSCanto II flow cytometers. For each run, donor specimens were stained with single-color fluorochrome-conjugated antibody reagents. Multiple sets of compensation tubes from multiple lots of BD FC beads were prepared by multiple operators. Analysis was performed using BD FACSDiva software v8.0.1 or later.

For each SOV, the absolute mean difference between the BD FC beads and the stained cells was calculated. Data analysis is shown in Table 1.

Table 1 Absolute mean difference in SOVs of BD FC beads vs stained cells

SOV	Absolute mean difference
FITC-%V500-C	0.66
PE-%FITC	0.06
PerCP-Cy5.5-%PE	1.39
PE-Cy7-%PerCP-Cy5.5	1.25
PE-%PE-Cy7	–0.77
APC-H7-%PE-Cy7	2.27
APC-%APC-H7	–2.19
APC-H7-%APC	0.32
V450-%V500-C	0.30
V500-C-%V450	0.62

Reproducibility

Multiple operators performed multiple runs per day over multiple days on multiple BD FACSCanto II flow cytometers. For each run, multiple sets of compensation tubes from multiple lots of BD FC beads were prepared. Analysis was performed using BD FACSDiva software v8.0.1 or later.

For each SOV, the reproducibility variance obtained from the BD FC beads was analyzed. Reproducibility was determined as two separate components. The first component (run/operator-to-run/operator, day-to-day, and lot-to-lot reproducibility) is shown in Table 2. The second component (instrument-to-instrument reproducibility) is shown in Table 3.

Table 2 Reproducibility (run/operator-to-run/operator, day-to-day, lot-to-lot)[a]

SOV	%CV[b]	UCL[c]
FITC-%V500-C	1.46	1.71
PE-%FITC	0.30	0.35
PerCP-Cy5.5-%PE	0.78	0.92
PE-Cy7-%PerCP-Cy5.5	0.32	0.37
PE-%PE-Cy7	6.84	8.01
APC-H7-%PE-Cy7	1.93	2.26
APC-%APC-H7	10.14	11.90
APC-H7-%APC	0.74	0.86
V450-%V500-C	0.70	0.82
V500-C-%V450	0.55	0.65

a. Degrees of freedom = 47
b. %CV = Percent coefficient of variation
c. UCL = Upper confidence limit of the 95% confidence interval

Table 3 Reproducibility (instrument-to-instrument)[a]

SOV	%CV	UCL
FITC-%V500-C	12.59	16.42
PE-%FITC	6.56	8.54
PerCP-Cy5.5-%PE	10.4	13.55
PE-Cy7-%PerCP-Cy5.5	2.42	3.15
PE-%PE-Cy7	6.71	8.72
APC-H7-%PE-Cy7	0	0
APC-%APC-H7	0.67	0.87
APC-H7-%APC	2.08	2.70
V450-%V500-C	15.87	20.74
V500-C-%V450	0.37	0.48

a. Degrees of freedom = 16

Repeatability

Compensation tubes of BD FC beads were prepared and acquired on multiple BD FACSCanto II flow cytometers. Analysis was performed using BD FACSDiva software v8.0.1 or later.

For each SOV, the repeatability variance obtained from the BD FC beads was analyzed. The repeatability is shown in Table 4.

Table 4 Repeatability[a]

SOV	%CV	UCL
FITC-%V500-C	1.32	1.51
PE-%FITC	0.90	1.03
PerCP-Cy5.5-%PE	1.03	1.18
PE-Cy7-%PerCP-Cy5.5	1.05	1.20
PE-%PE-Cy7	1.41	1.62
APC-H7-%PE-Cy7	1.16	1.33
APC-%APC-H7	1.62	1.85
APC-H7-%APC	1.34	1.54
V450-%V500-C	0.97	1.11
V500-C-%V450	0.94	1.08

a. Degrees of freedom = 64

WARRANTY

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TROUBLESHOOTING

Problem	Possible Cause	Solution
Compensation calculation is not successful	Gates are not properly adjusted	1. Adjust the gates to include the appropriate bead populations. 2. Recalculate compensation.
	BD FC beads are expired	Prepare new bead tubes from a current lot, then rerun compensation setup.
	Rehydrated bead tubes are exposed to light or used beyond the stability period	Prepare new bead tubes, then rerun compensation setup.
	Cytometer fluidics problem	Check cytometer fluidics for bubbles or debris. See the cytometer IFU for information.
No beads detected	Pouch not resealed properly	Open a new pouch, or use tubes from a pouch that was resealed properly.
	FSC and SSC PMTVs not optimum for beads	Optimize FSC and SSC PMTVs.
	Air bubbles in the flow cell or sheath filter	Check cytometer fluidics for bubbles or debris. See the cytometer IFU for information.
	Clogs within the sample tubing and lines	Check the fluidics for clogs and debris. See the cytometer IFU for information.
	Back pressure in the waste lines	Check the waste tank vent for obstructions. See the cytometer IFU for information.
	High scatter noise (FSC or SSC)	Perform monthly maintenance. See the cytometer IFU for information. Call BD Biosciences.