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

5 tests per kit—Catalog No. 658621

23-15785(02)
2023-04
English

CE IVD

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 OF THE TEST

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.

The BD® FC Beads 8-Color Kit for BD OneFlow™ Assays is intended for use by laboratory professionals.

Principle of Operation

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 are used to perform daily cytometer quality control. 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.

3. REAGENT

Reagent Composition

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.

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.

The beads contain 0.81% 2-methyl-4-isothiazolin-3-one (CAS number 2682-20-4, EC number 220-239-6). The beads are classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and Regulation (EC) No 1272/2008. Go to regdoc.bd.com/regdocs/sdsSearch to download the Safety Data Sheet.

	Warning
	H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects.
Prevention	P261: Avoid breathing dust/fume/gas/mist/vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves/protective clothing/eye protection/face protection. P273: Avoid release to the environment.
Response	P302+P352: IF ON SKIN: Wash with plenty of soap and water. P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P362+P364: Take off contaminated clothing and wash it before reuse.
Disposal	P501: Dispose of contents/container to an approved facility in accordance with local, regional, national and international regulations.

Storage and Handling

- Store tubes at 2–8 °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 2–8 °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 2–8 °C

4. 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 BD FACSDiva™ CS&T IVD Beads
- After cytometer maintenance or service is performed

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-Cy5.5
  – PE-Cy7
  – 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 (Catalog No. 656046 or 656047)

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

  See the *BD OneFlow™ Setup Beads* IFU.

Preparing BD® FC Beads

CAUTION Before preparing BD® FC Beads, verify that the:

- 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™ Setup Beads* IFU

1. Allow the bead pouches to reach 18–25 °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 2–8 °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 bead 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 2–8 °C protected from light. After rehydration, the beads are stable for:

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

Creating compensation controls

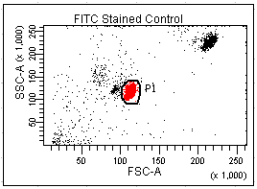
1. In the title bar of the BD FACSDiva™ workspace, confirm that the 4-2H-2V optical configuration is selected.
2. From the menu bar, select **Experiment > New Experiment > Blank Experiment > OK**.
3. If prompted by the CST Mismatch dialog, select **Use CST Settings**.
4. In the Browser, right-click the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

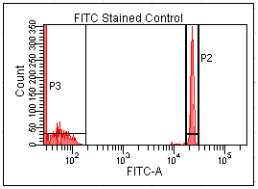


NOTE Increase the FSC PMTV to resolve the singlet bead population.

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

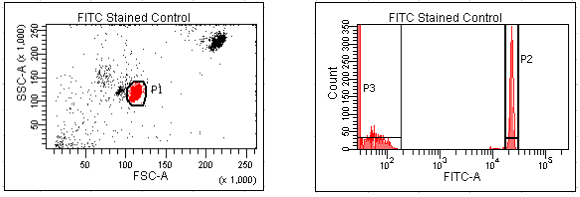


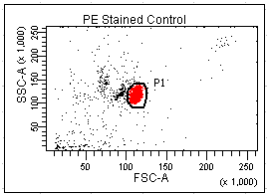
11. Select the next tube.
12. Repeat steps 1 through 11 for the remaining fluorochrome tubes. See Figure 2 for representative data.
13. From the menu bar, select **Experiment > Compensation Setup > Calculate Compensation**.
14. Name the compensation file with the run date appended with OneFlow (for example,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

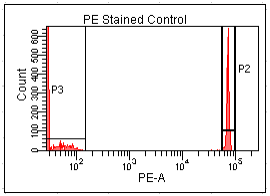
5. REPRESENTATIVE DATA

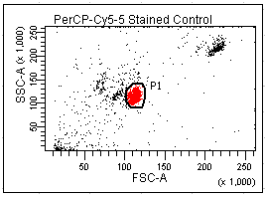
Figure 2 displays representative BD® FC Beads data acquired and analyzed on a BD FACSCanto™ II flow cytometer with a 4-2H-2V optical configuration and BD FACSDiva™ software. Laser excitation is at 488 nm (FITC, PE, PerCP-Cy5.5, PE-Cy7), 640 nm (APC, APC-H7), and 405 nm (V450, V500-C). Detector voltages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

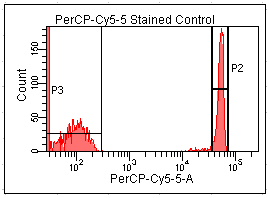
Figure 2 Representative data

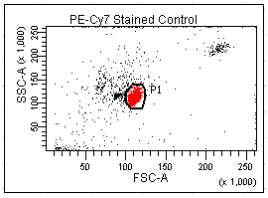


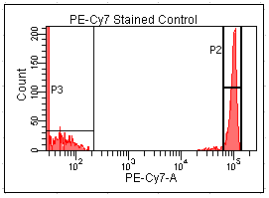

PE Stained Control
SSC-A (x 1,000)
FSC-A (x 1,000)
P1

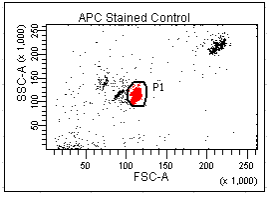

PE Stained Control
Count
PE-A
P2
P3

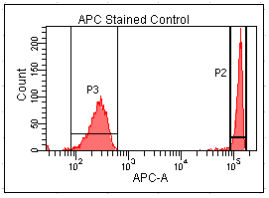

PerCP-Cy5-5 Stained Control
SSC-A (x 1,000)
FSC-A (x 1,000)
P1

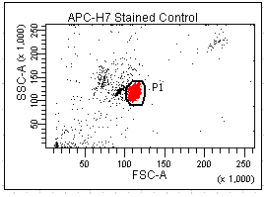

PerCP-Cy5-5 Stained Control
Count
PerCP-Cy5-5-A
P2
P3

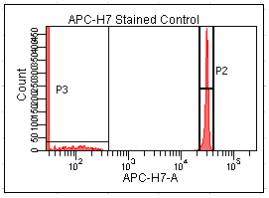

PE-Cy7 Stained Control
SSC-A (x 1,000)
FSC-A (x 1,000)
P1

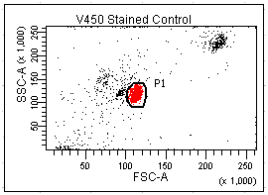

PE-Cy7 Stained Control
Count
PE-Cy7-A
P2
P3

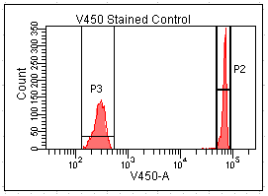

APC Stained Control
SSC-A (x 1,000)
FSC-A (x 1,000)
P1

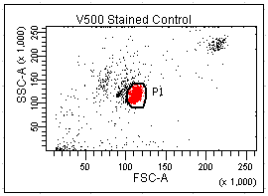

APC Stained Control
Count
APC-A
P2
P3

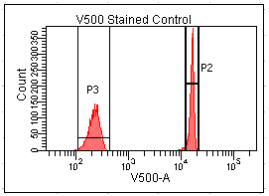

APC-H7 Stained Control
SSC-A (x 1,000)
FSC-A (x 1,000)
P1


APC-H7 Stained Control
Count
APC-H7-A
P2
P3









6. 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.

7. 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

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

SOV	Absolute mean difference
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 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

8. 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.

NOTICE

EU Only: Users shall report any serious incident related to the device to the Manufacturer and National Competent Authority.

Outside EU: Contact your local BD representative for any incident or inquiry related to this device.

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 SOLE 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.

PATENTS AND TRADEMARKS

For US patents that may apply, see bd.com/patents.

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HISTORY

Revision	Date	Changes made
23-15785(01)	2022-04	Updated to meet requirements for Regulation (EU) 2017/746.
23-15785(02)	2023-04	Updated legal manufacturer address. Added EU and Swiss importer addresses. Updated symbols glossary.

SYMBOLS GLOSSARY

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Please refer to product labeling for applicable symbols.

Symbol	Meaning
	Manufacturer
EC REP	Authorized representative in the European Community
CH REP	Authorised representative in Switzerland
	Date of manufacture
	Use-by date
LOT	Batch code
REF	Catalogue number
SN	Serial number
STERILE	Sterile
STERILE A	Sterilized using aseptic processing techniques
STERILE EO	Sterilized using ethylene oxide
STERILE R	Sterilized using irradiation
STERILE	Sterilized using steam or dry heat
	Do not resterilize
NON PYROGENIC	Non-sterile
	Do not use if package is damaged and consult *instructions for use*
STERILE	Sterile fluid path
STERILE EO	Sterile fluid path (ethylene oxide)
STERILE R	Sterile fluid path (irradiation)
	Fragile, handle with care
	Keep away from sunlight
	Keep dry
	Lower limit of temperature
	Upper limit of temperature
	Temperature limit
%	Humidity limitation
	Biological risks
	Do not re-use
	Consult *instructions for use* or consult electronic *instructions for use*
	Caution
LATEX	Contains or presence of natural rubber latex
IVD	In vitro diagnostic medical device
CONTROL -	Negative control
CONTROL +	Positive control
n	Contains sufficient for *<n>* tests
	For IVD performance evaluation only
	Non-pyrogenic
#	Patient number
	This way
	Do not stack

Symbol	Meaning
	Single sterile barrier system
PHT DEHP BBP	Contains or presence of phthalate: combination of bis(2-ethylhexyl) phthalate (DEHP) and benzyl butyl phthalate (BBP)
	Collect separately Indicates separate collection for waste of electrical and electronic equipment required.
CE	CE marking; Signifies European technical conformity
	Device for near-patient testing
	Device for self-testing
Rx Only	This only applies to US: "Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner."
	Country of manufacture "CC" shall be replaced by either the two letter or the three letter country code.
	Collection time
	Cut
	Peel here
	Collection date
	Keep away from light
	Hydrogen gas is generated
	Perforation
	Start panel sequence number
	End panel sequence number
	Internal sequence number
i	<Box #> / <Total Boxes>
MD	Medical device
	Contains hazardous substances
	Ukrainian conformity mark
FC	Meets FCC requirements per 21 CFR Part 15
c UL us	UL product certification for US and Canada
UDI	Unique device identifier
	Importer
	Place patient label in framed area only
MR	Magnetic resonance (MR) safe
MR	Magnetic resonance (MR) conditional
MR	Magnetic resonance (MR) unsafe
For use with	For use with
This Product Contains Dry Natural Rubber	This Product Contains Dry Natural Rubber
For Export Only	For Export Only
Instruments	Instruments

Note: Text layout in symbols is determined by label design.

L006715(08) 2023-03

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