BD™ FC Beads

Form	Catalog number	Form	Catalog number	Form	Catalog number
FITC	661615	APC	661620	BV605[a]	661626
PE	661616	APC-Cy7	661622	BV421[a]	661627
PerCP-Cy™5.5	661619	APC-H7	661621	BV510[a]	661628
PerCP	661618	APC-R700[a]	661625	BV711[a]	661629
PE-Cy™7	661617	V450[a]	661623	BV786[a]	661630
BB515[a]	661631	V500-C[a]	661624		

a. BD Horizon Brilliant™ Blue 515, BD Horizon™ APC-R700, BD Horizon™ V450, BD Horizon™ V500-C, BD Horizon Brilliant™ Violet 605, BD Horizon Brilliant™ Violet 421, BD Horizon Brilliant™ Violet 510, BD Horizon Brilliant™ Violet 711, BD Horizon Brilliant™ Violet 786

DESCRIPTION

BD™ FC Beads are fluorescent beads that enable the software to determine spillover values (SOVs) for fluorescence compensation and to calculate a fluorescence compensation matrix during setup of BD flow cytometers. The combination of BD™ CS&T RUO Beads and BD FC Beads sets the voltage and compensation for each of the channels of the instrument. BD FC Beads can also be used to standardize multiple BD flow cytometer instruments.

MATERIALS

BD FC Beads are 3-µm polystyrene beads coupled to fluorochromes and dried down in single-use 12 × 75-mm tubes. Each tube comprises a mixture of positive beads and negative beads.

Reagent provided

The beads contain dyes that will compensate for the fluorochromes listed. Five tubes of beads for a particular fluorochrome are provided desiccated in a resealable foil pouch.

Reagents and materials required but not provided

- BD™ FC Beads Dilution Buffer (Catalog No. 661614)
- Vortex mixer

HANDLING AND STORAGE

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 dilution buffer are stable until the expiration date shown on the pouch and bottle labels when stored as directed. Do not use after the expiration date.

NOTE Reseal the pouch and return it to 2°C–8°C storage immediately. The reagent is very sensitive to moisture. Do not remove the desiccant pack from the pouch.

Some of the dyes used to manufacture the beads are very light sensitive. Fluorescence spillover values can change if the beads are exposed to light.

SUPPORTED INSTRUMENTS

Automatic calculation of spillover using BD FC Beads can be performed on the following BD instruments:

Instrument	Software
BD FACSMelody™ cell sorter	BD FACSChorus™ software
BD FACSLyric™ flow cytometer	BD FACSuite™ software

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NOTE In the United States, the BD FACSLyric flow cytometer is cleared for in vitro diagnostic use on six channels. Use BD FC Beads 7-Color Kit (Catalog No. 662961) to calculate compensation for the six IVD channels. In Europe and other regions where the BD FACSLyric flow cytometer is CE-marked and designated for in vitro diagnostic use, use BD™ FC Beads 7-Color Kit (Catalog No. 656867) and BD™ FC Beads 5-Color Kit (Catalog No. 661564) to calculate compensation.

PROCEDURE

Downloading bead lot files

Before using a new lot of BD FC Beads, download the appropriate bead lot file. The information in the file is used by the instrument's software.

To download the bead lot file:

1. Visit bdbiosciences.com and click **Support** from the menu bar.

   The **Services** web page opens.

2. Select **Bead Lot Files:** for the appropriate instrument or software under **Top Support Links** in the right panel and navigate to the **Bead Lot File** web page, as needed.

3. Follow the installation instructions on the website to download and import the appropriate bead lot file into the software.

   For the BD FACSLyric flow cytometer, ensure that the bead lot file you download is for the appropriate BD FC Beads and corresponds to your current lot of beads. The lot number is found on the pouch label.

   For the BD FACSMelody cell sorter, run the **BD FACSChorus FC Bead Lot Updater**, as needed. See the *BD FACSMelody™ Cell Sorter User's Guide* for more information.

   NOTE Bead lot files are not needed when using single-color BD FC Beads to set up and standardize instruments.

Preparing the beads

To prepare the BD FC Beads:

1. Allow the bead pouches to reach room temperature, 18°C–25°C.
2. When using the beads to calculate compensation, confirm that the Lot ID on the pouch label matches the Lot ID in the software or bead lot file.

   NOTE Bead lot files are not needed when using the beads to set up and standardize instruments.

3. Open a pouch, remove one tube, and place it in a rack, protected from light.
4. Reseal the pouch and return it to 2°C–8°C storage immediately. Do not remove the desiccant pack from the pouch.
5. Repeat steps 2 and 3 for the remaining tubes that you want to use.
6. Add 0.5 mL (10 drops) of BD FC Beads Dilution Buffer to each tube.

   NOTE Use of other dilution buffers could result in incorrect SOVs.

7. Vortex the tubes vigorously for 3–5 seconds to rehydrate the beads.
8. Store the rehydrated beads at 2°C–8°C, protected from light, if not using immediately.

   After rehydration, when protected from light, the beads are stable for:

   – 1 hour at 18°C–25°C

   – 4 hours at 2°C–8°C

Calculating compensation

To calculate compensation:

1. Vortex each tube 3–5 seconds immediately before acquiring it.
2. Install the tube on the instrument.
3. Follow the prompts in the software to calculate fluorescence compensation.

   See the user's guide for your instrument for more information.

Standardizing multiple instruments

In addition to being used to calculate fluorescence compensation, BD FC Beads can be used to standardize multiple BD flow cytometer instruments.

Additional reagents and materials required but not provided

- 12 × 75-mm polystyrene tubes
- Lysed washed blood

  You will need unstained and stained lysed washed blood. Visit our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.
- BD FACS™ Lysing Solution (Catalog No. 349202)

  See the *BD FACS™ Lysing Solution* instructions for use for precautions and dilution instructions.
- Fluorochrome-conjugated CD4 antibodies

  You will need an antibody for each fluorochrome that you intend to set up.

  CD4 is a well established reference marker. However, you can substitute a fluorochrome-conjugated antibody for any CD marker.

Preparing the beads

Prepare the beads as described previously.

Standardizing the instruments

To determine the target values:

1. From the pool of instruments designated for setup, review their respective QC reports, such as the Cytometer Setup Report or Cytometer Characterization QC Report, and determine the instrument with the highest electronic noise (Electronic Noise RSD).

   Use this instrument to determine the initial target values to be used for the alignment.
2. Acquire unstained lymphocytes at a low flow rate.
3. Adjust the photomultiplier tube (PMT) voltage settings for the detectors such that the robust standard deviation (rSD) of the unstained lymphocytes is 2.5 times higher than the rSD of the electronic noise.

   This ensures that contribution of electronic noise to the SD of the unstained population is less than 10%.
4. Acquire CD4-stained lymphocytes.
5. Reduce the PMT voltages (PMTVs), if necessary, so that the median fluorescence intensity (MFI) of the CD4-positive population is within the linear range for each detector.
6. Vortex each tube of beads 3–5 seconds immediately before acquiring it.

7. Acquire each tube of beads.

   Record the MFI of the beads in each channel and save them as target values to be used for instrument setup using BD FC Beads.

To standardize the instruments using BD FC Beads:

1. Vortex each tube of beads 3–5 seconds immediately before acquiring it.
2. Acquire each tube of beads on each instrument.
3. Set the PMTVs such that the MFIs of the positive bead populations are at the target values established on the instrument identified previously as having the highest electronic noise.
4. Record 5,000 events.
5. Acquire the CD4-stained lymphocytes for each fluorochrome and record 5,000 events.

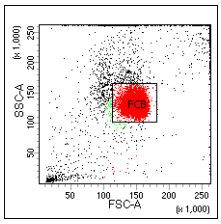
Analyzing the data

To analyze the data for instrument setup using BD FC beads:

1. For each fluorochrome, determine the ratio (R) of the MFI of the CD4-positive cells to the MFI of the positive beads population.
2. Perform this for all of the instruments.
3. Calculate the coefficient of variation (CV) of the ratios obtained from different instruments.
4. (Optional) Calculate the greatest difference between instruments by determining the percent difference of the two ratios with the largest difference between them.
5. Plot the MFI of the CD4-positive lymphocytes across the different instruments.

REPRESENTATIVE DATA

The plot shows BD FC Beads acquired on a BD flow cytometer. For each bead, draw a gate around the singlet population in the FSC-A vs SSC-A dot plot. The events inside the FCB gate include both the positive beads and negative beads for the specified fluorochrome. Gate on both the positive beads and the negative beads in the appropriate histogram when calculating compensation.



WARNING

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[1,2] and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

The beads contain 0.8125% 2-methyl-4-isothiazolin-3-one (CAS number 2682-20-4) and either 0.0624% or 0.0627% sodium azide (CAS number 26628-22-8). The beads are classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

	Warning
	H317 May cause an allergic skin reaction. H402 Harmful to aquatic life. 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. P302+P352 IF ON SKIN: Wash with plenty of water. P333+P313 If skin irritation or rash occurs: Get medical advice/attention. P321 Specific treatment (see Safety Data Sheet). P363 Wash contaminated clothing before reuse. P501 Dispose of contents/container to an appropriate treatment and disposal facility in accordance with applicable laws and regulations, and product characteristics at time of disposal.

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.

REFERENCES

1. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document M29-A4.
2. Centers for Disease Control. Perspectives in disease prevention and health promotion update: universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens in health-care settings. *MMWR*. 1988;37:377-388.

PATENTS AND TRADEMARKS

BV421, BV510, BV605, BV711, BV786, and V500-C are covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,227,187; 8,455,613; 8,362,193; 8,575,303; 8,354,239; or 8,431,416.

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