BD™ FC Beads

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

a.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™ Blue 515

DESCRIPTION

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

MATERIALS

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

Reagent provided

BD FC beads contain dyes that will compensate for the fluorophores listed. Five tubes of each BD FC bead 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

BD FC beads can be used on any BD™ instrument designated for research use, running the appropriate BD™ software. See the User's Guide for your instrument for more information.

PROCEDURE

CALCULATING COMPENSATION

Preparing the beads

To prepare the BD FC beads:

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

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3. Reseal the pouch and return it to 2°C–8°C storage immediately. Do not remove the desiccant pack from the pouch.
4. Repeat steps 2 and 3 for the remaining tubes that you want to use.
5. 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.
6. Vortex the tubes vigorously for 3–5 seconds to rehydrate the beads.
7. 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 8°C–25°C

   – 4 hours at 2°C–8°C

Calculating fluorescence 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.

SETTING UP MULTIPLE INSTRUMENTS

In addition to being used to calculate fluorescence compensation, BD FC beads can be used to set up and standardize multiple instruments.

Additional reagents and materials required but not provided

- BD™ CS&T beads
- BD FACSFlow™ sheath fluid
- 12 × 75-mm polystyrene tubes
- Lysed washed blood

  You will need unstained and stained lysed washed blood. Lyse whole blood using BD FACS™ lysing solution. Visit our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.
- BD FACS lysing solution
- 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 BD FC beads as described previously.

To prepare the BD CS&T beads:

1. Add 0.5 mL BD FACSFlow sheath fluid to a 12 × 75-mm polystyrene tube.
2. Vortex the vial of BD CS&T beads at medium speed for 5–10 seconds.
3. Add 2 drops of BD CS&T beads to the tube.
4. Vortex 3–5 seconds to mix.

Standardizing the instruments

To set up the target values:

1. From the pool of instruments designated for setup, review their respective CS&T baseline reports and determine the instrument with the highest electronic noise (SDen).

   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 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. Acquire the BD CS&T beads.

   Record the MFI of the beads in each channel and save them as target values to be used for CS&T setup.
7. Acquire each tube of the BD FC beads.

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

Setting up the instruments

Perform CS&T setup:

1. Acquire BD CS&T beads on each instrument.
2. Set the PMTVs such that the bright bead MFIs for each channel are at the target values established on the first instrument.
3. Record 30,000 events.
4. Acquire the CD4-stained lymphocytes for each fluorochrome and record 5,000 events.

Perform FC beads setup:

1. Acquire each tube of BD FC beads on each instrument.
2. Set the PMTVs such that the MFIs of the positive bead populations are at the target values established on the first instrument.
3. Record 5,000 events.
4. Acquire the CD4-stained lymphocytes for each fluorochrome and record 5,000 events.

Analyzing the data

Analyze the data for setup using BD CS&T beads:

1. Determine the ratio (R) of the MFI of the CD4-positive cells to the MFI of the CS&T bright beads for each detector.
2. Perform this for all of the instruments being aligned.
3. Calculate the CV of the ratios.

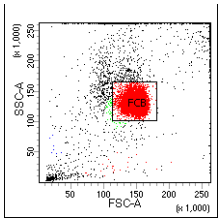
Analyze the data for setup using BD FC beads:

1. Determine the ratio (R) of the MFI of the CD4-positive cells to the MFI of the CS&T bright beads for each detector.
2. Perform this for all of the instruments.
3. Calculate the CV of the ratios.
4. Plot the MFI of the CD4-positive lymphocytes across the different instruments.

REPRESENTATIVE DATA

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 fluorophore. 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% of 2-methyl-4-isothiazolin-3-one (CAS number 2682-20-4) and 0.0627% of sodium azide (CAS number 26628-22-8).

Warning

May cause an allergic skin reaction. Harmful to aquatic life.

Avoid breathing dust/fume/gas/mist/vapors/spray. Contaminated work clothing must not be allowed out of the workplace. Wear protective gloves, protective clothing/eye protection/face protection. Avoid release to the environment.

IF ON SKIN: Wash with plenty of water. If skin irritation or rash occurs: Get medical advice/attention. Specific treatment (see Safety Data Sheet). Wash contaminated clothing before reuse.

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.

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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.

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