BD™ Cytometer Setup and Tracking Beads

One 3-mL vial—Catalog No. 641319
Three 3-mL vials—Catalog No. 642412

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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1. USAGE

BD™ Cytometer Setup and Tracking beads are for research use only with BD FACSDiva™ software (v6.x). The beads allow the software to automatically characterize, track, and report measurements of supported BD digital flow cytometers.

Each vial of beads contains equal concentrations of beads of three fluorescence emission intensities: bright, mid, and dim. The beads are used to define a baseline and run daily measurements of the cytometer. Each 3-mL vial contains beads sufficient for 50 daily measurements or 16 baseline definitions.

Cytometer Setup and Tracking beads consist of bright (3-µm), mid (3-µm), and dim (2-µm) beads dyed with a mixture of fluorochromes that are excited by the lasers used in BD digital flow cytometers. The beads emit fluorescence in detectors used for the following fluorochromes:

Fluorochromes	Excitation Laser (nm)	Emission Range (nm)
Indo 1, DAPI, Hoechst	UV 355 and 375	400–550
Pacific Blue™[a], AmCyan, Qdot 655, Qdot 700, Alexa Fluor®[a] 405	violet 405 and 407	420–700
FITC, PE, PE-Texas Red®[a], PerCP, PerCP-Cy™5.5[b], PE-Cy™7[bc]	blue 488	500–800
PE, PE-Texas Red®, PerCP, PerCP-Cy5.5, PE-Cy7	green 532	550–800
APC, APC-Cy7[c], APC-HL 750, Alexa Fluor® 700	red 633 and 645	650–800

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b. Cy™ is a trademark of GE Healthcare. This product is subject to proprietary rights of GE Healthcare and Carnegie Mellon University, and is made and sold under license from GE Healthcare. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one, return this material, unopened, to BD Biosciences, 2350 Qume Drive, San Jose, CA 95131, and any money paid for the material will be refunded.

c. Patents—APC-Cy7: US 5,714,386

First, Cytometer Setup and Tracking beads are used to define a cytometer baseline.[1] Diluted beads are run on the flow cytometer using BD FACSDiva software. Median fluorescence intensity (MFI) and robust CV (rCV) are measured for each bead intensity in all fluorescence detectors. Algorithms within the software differentiate the fluorescence signal from each bead type based on size and fluorescence intensity in each detector. The software then uses this data to calculate and report a variety of measurements. These measurements include relative fluorescence detection efficiency (Qr), relative background (Br), the standard deviation of electronic noise, and cytometer settings adjusted for maximizing population resolution in each detector.[2–6]

Once baseline values are defined, the beads are used to run daily measurements to reproducibly set up the cytometer from day to day. Application settings associated with cytometer configurations in BD FACSDiva software are automatically updated. Daily measurements are automatically entered into Levey-Jennings plots, allowing you to monitor these cytometer measurements and detect potential problems.

2. REAGENTS

Cytometer Setup and Tracking beads consist of equal concentrations of 3-µm bright, 3-µm mid, and 2-µm dim polystyrene beads in phosphate buffered saline (PBS) with bovine serum albumin (BSA), and sodium azide in a stream-tip dropper vial.

Precautions

- Cytometer Setup and Tracking beads are For Research Use Only.
- Cytometer Setup and Tracking beads solution contains sodium azide as a preservative. Use care to avoid microbial contamination, which can cause erroneous results.
- **WARNING** All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[7,8] 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.
- **WARNING** Do not dilute Cytometer Setup and Tracking beads more than recommended. Doing so will generate an error while characterizing baseline, running daily measurements, and resetting target values.

Storage and Handling

- Store the beads vial at 2°C–8°C and protect from light.
- Do not freeze Cytometer Setup and Tracking beads.
- Vial contents are stable for the period shown on the vial label when stored as directed. Do not use after the expiration date.
- Cytometer Setup and Tracking beads can be diluted in BD FACSFlow™ solution, BD FACSFlow solution with surfactant, or PBS. (See Procedure.) For consistent results, we recommend always using the same diluent and sample delivery device to run the Cytometer Setup and Tracking beads.

- After dilution, the beads suspension is stable for 8 hours at 2°C–25°C when protected from light.

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

3. SUPPORTED CYTOMETERS

Cytometer Setup and Tracking beads are supported on the BD FACSCanto™ (for research applications only), BD FACSAria™, and BD™ LSR II digital flow cytometer platforms. The cytometer workstation must be equipped with BD FACSDiva software v6.x. Refer to your cytometer user's guide, the *BD FACSDiva Getting Started Guide,* and the *BD Cytometer Setup and Tracking Application Guide* for more information.

4. PROCEDURE

For detailed instructions and troubleshooting information, refer to the *BD Cytometer Setup and Tracking Application Guide*.

Optimization of cytometer settings for applications using stained biological samples might be required following cytometer setup.

Prepare the Cytometer Setup and Tracking beads suspension immediately before use.

Materials Required but Not Provided

- Disposable 12 x 75-mm Falcon®* capped polystyrene tubes (BD Catalog No. 352058), or equivalent
- Vortex mixer
- BD FACSFlow solution, BD FACSFlow solution with surfactant, or PBS
- BD FACS™ digital flow cytometer

  Refer to the appropriate cytometer manual for operating instructions.

- BD FACSDiva software, v6.x, for cytometer setup

  Refer to the *BD Cytometer Setup and Tracking Application Guide* for setup information.

- Multiwell plates

*Falcon is a registered trademark of Corning Incorporated.

Running a New Lot of Beads

Before running a new lot of beads, go to bdbiosciences.com and download the lot-specific data file to your desktop. Refer to the *BD Cytometer Setup and Tracking Application Guide* for instructions on how to import the lot-specific information into BD FACSDiva software. The file will be used by the software to normalize cytometer tracking for BD-defined filters.

Preparing Cytometer Setup and Tracking Beads in Tubes

1. Label a 12 x 75-mm capped polystyrene tube *setup beads*.
2. Mix the bead vial by gentle inversion or very gentle vortexing.
3. Prepare the beads suspension.
   - **For defining baseline**, add to the labeled tube:
     - 0.5 mL diluent
     - 3 drops of beads
   - **For running daily measurements**, add to the labeled tube:
     - 0.35 mL diluent
     - 1 drop of beads
   - **For resetting target values**
     - add to a tube labeled *current lot*:
       - 0.5 mL diluent
       - 3 drops of beads from current lot
     - add to a tube labeled *new lot*:
       - 0.5 mL diluent
       - 3 drops of beads from new lot
4. Vortex the tube gently before use.

   Store the beads suspension at 2°C–25°C in the dark if not using immediately.

Preparing Cytometer Setup and Tracking Beads in Plates

1. Mix the Cytometer Setup and Tracking beads vial by gentle inversion or very gentle vortexing.
2. Add the following to each of the specified wells of a multiwell plate:
   - **For defining baseline**, add to wells A1 through A4:
     - 150 µL diluent
     - 1 drop of beads
     - **For running daily measurements**, add to well A1:
     - 150 µL diluent
     - 1 drop of beads
   - **For resetting target values**
     - add to well A1:
       - 150 µL diluent
       - 1 drop of beads from current lot
     - add to well A2:
       - 150 µL diluent
       - 1 drop of beads from new lot

   Store the beads suspension at 2°C–25°C in the dark if not using immediately.

5. DATA ACQUISITION AND ANALYSIS

For detailed instructions on establishing baseline values and running daily measurements using BD FACSDiva software

v6.x, refer to the *BD Cytometer Setup and Tracking Application Guide*.

The following figures show representative Cytometer Setup and Tracking beads data analyzed on a BD FACS digital flow cytometer with laser excitation at 488 nm using BD FACSDiva software.

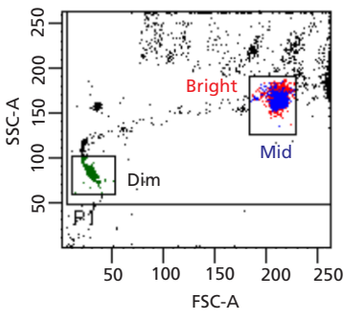

Figure 1 Dot plot showing Cytometer Setup and Tracking beads

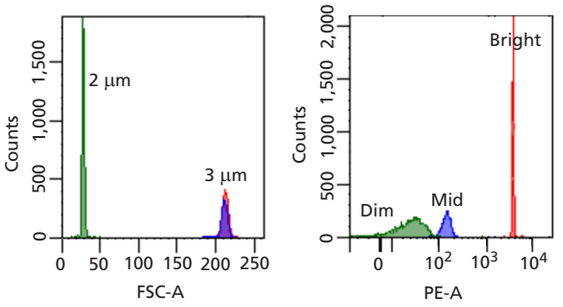

Figure 2 Cytometer Setup and Tracking beads histograms showing bead size and separation

LIMITATIONS

- Because some of the dyes used to manufacture the beads are very light sensitive, protect the beads from light. Fluorescence levels can change if beads are exposed to direct light longer than 20 minutes.
- Bead performance might vary depending on laser and filter combinations.
- For consistent results, we recommend always using the same diluent and sample delivery device to run the beads.

TROUBLESHOOTING

Refer to the *BD Cytometer Setup and Tracking Application Guide* for troubleshooting information.

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. 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, ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING PERSONAL INJURY, OR ECONOMIC LOSS CAUSED BY THE PRODUCT.

REFERENCES

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CUSTOMER SUPPORT INFORMATION

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PATENTS AND TRADEMARKS