Creating and Saving Optimal Settings in BD FACSuite Software

Run CQC and PQC

⬇

Determine 2.5 x rSD_{EN} with unstained cells

⬇

Verify stained cells within the linearity of each parameter

⬇

Save optimal settings and create an assay

⬇

Transfer the assay to remaining cytometers

Reusing optimal settings in BD FACSuite software

Run PQC

⬇

Run Assay and Tube Settings Setup

⬇

Run Assay in Worklist or Create Experiment from Assay

⬇

Acquire Data

Note: If reference settings were created during the initial optimization, they will need to be updated every 30 days.

BD Biosciences Regional Offices

Australia
Toll Free 1800 656 100
Tel 61.2.8875.7000
Fax 61.2.8875.7200
bdbiosciences.com/anz

Canada
Tel 866.979.9408
Fax 888.229.9918
bdbiosciences.com/ca

China
Tel 86.21.3210.4610
Fax 86.21.5292.5191
bdbiosciences.com/cn

Europe
Tel 32.2.400.98.95
Fax 32.2.401.70.94
bdbiosciences.com/eu

India
Tel 91.124.2383566
Fax 91.124.2383224/25/26
bdbiosciences.com/in

Japan
Nippon Becton Dickinson
Toll Free 0120.8555.90
Fax 81.24.593.3281
bd.com/jp

Latin America/ Caribbean
Toll Free 0800.771.71.57
Fax 55.11.5185.9688
bdbiosciences.com/br

New Zealand
Toll Free 0800 572.468
Tel 64.9.574.2468
Fax 64.9.574.2469
bdbiosciences.com/anz

Singapore
Tel 65.6861.8691
Fax 65.6860.1593
bdbiosciences.com/sg

United States
US Orders 855.236.2772
Technical Service
877.232.8995
Fax 800.325.9637
bdbiosciences.com

Office locations are available on our websites.

23-16366-00

BD FACSuite™ Software

Standardizing Application Settings Across Multiple Cytometers

Multicolor flow cytometry is increasingly used in research laboratories to generate more data from a given experiment.

A key aspect of effectively using multicolor flow is ensuring that the experiment can be performed consistently over time and across instruments. Standardizing experiments using application setup can ensure consistency of results over time across multiple systems. To achieve this consistency, we recommend creating and transferring Assays in BD FACSuite software.

To create and save the optimal settings for specific experiments, follow these basic steps:

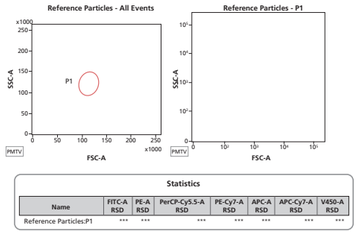
Step 1: Run Characterization and Performance QC
Step 2: Determine 2.5 x rSD_{EN} with unstained cells
Step 3: Verify stained cells within the linearity of each parameter
Step 4: Save optimal settings and create an assay
Step 5: Transfer the assay to remaining cytometers

Step 1: Run Characterization QC (CQC) and Performance QC (PQC)

a. Verify that CQC has been run.
b. Run PQC on each cytometer.
c. Record the rSD_{EN} and linearity max for each fluorescence channel for each cytometer.

Step 2: Determine 2.5 x rSD_{EN} with unstained cells

a. Multiply the rSD_{EN} by 2.5 for all fluorescence channels.
b. Compare these values across all cytometers and record the highest value for each channel.
c. Adjust the PMT voltage (PMTV) for each channel until the rSD for unstained cells reaches this value.

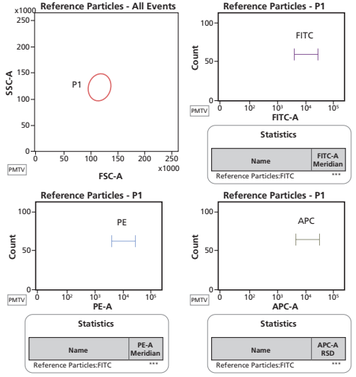


An example of a worksheet used to acquire unstained cells and adjust PMTVs until the target rSD values (2.5 x rSD_{EN}) are reached.

Step 3: Verify stained cells within the linearity of each parameter

a. Divide the linearity max value by 2 for all fluorescence channels.
b. Compare these values across all cytometers and record the lowest value for each channel.
c. Using highly positive cell populations, verify that the median fluorescence intensity (MFI) of the positive population falls at or below this number.

Note: If the MFI exceeds this value for any channel, lower the PMTV.



An example of a worksheet used to verify that MFI values for stained cells fall within the linearity of each parameter.

Step 4: Save optimal settings and create an Assay

a. Create tube settings or reference settings by right-clicking the tube and selecting **Create Tube Settings** or **Create Reference Settings**.
b. Create an assay by selecting **File** > **Create Assay**.

Step 5: Transfer settings to the remaining cytometers

a. Export your assay from the first cytometer. In the **User-Defined Assays** section of the Library, select your assay, then click **File** > **Export**.
b. Import your assay to the remaining cytometers. In the **User-Defined Assays** section of the Library, select **File** > **Import**.
c. Run assay and tube setting setup for the appropriate assay. In the **Setup & QC** workspace, select your assay from the **Assay & Tube Settings Setup** task menu, then click **Start**.