

Setup Procedure for BD Accuri™ C6

This part of the guide applies to BD digital flow cytometers using BD Accuri™ C6 software.

For the BD Accuri™ C6, use the PE channel (FL2) for the reporter and the APC channel (FL4) for beads classification. The Setup Beads 3: Raw Beads are used to setup the machine. Setup Beads 1: FITC Beads and 2: PE Beads are not needed for this setup because no compensation is required if the setup procedure described here is closely followed.

This setup procedure is required under the following situations:

- You are running the LEGENDplex™ kit for the first time.
- It has been over a month since the procedure was last performed.
- Your flow cytometer has been serviced since you last performed this procedure.

This setup procedure is not needed if you have run the setup procedure before and have access to a saved template (see step 2. below).

1. Start up the Instrument

Perform instrument startup and verification check following the manufacturer's recommendations.

2. Creating a new template

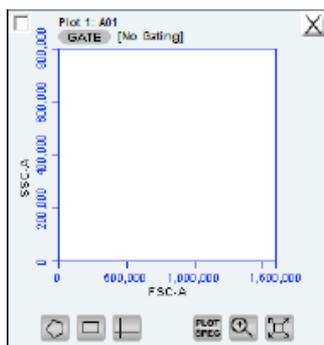
A template for BD Accuri™ is a workspace with density plots and proper machine settings that allows the user to perform data acquisition.

If a template is not yet available, create a new template following the instructions below. After a template is created, save in a designated location where the template is easily accessible.

2.1 From the BD Accuri™ C6 software, go to new Workspace.

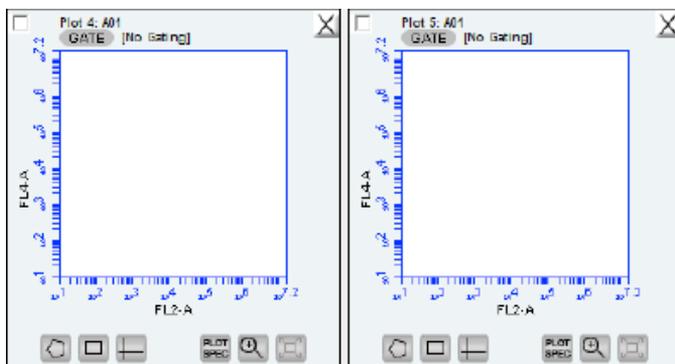
2.2 In the workspace, you can see an automatically created dot plot with FSC (forward scatter) for X-axis and SSC (side scatter) for Y-axis. **Be sure to set FSC and SSC to linear mode** (Figure 19).

Figure 19.



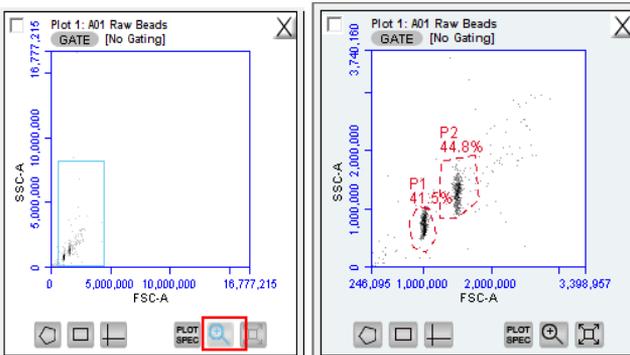
- 2.3 Create two dot plots (Figure 20, Plot 4 and Plot 5) with PE (FL2) for X-axis, APC (FL4) for Y-axis. **The plots should all be in log mode.**

Figure 20.



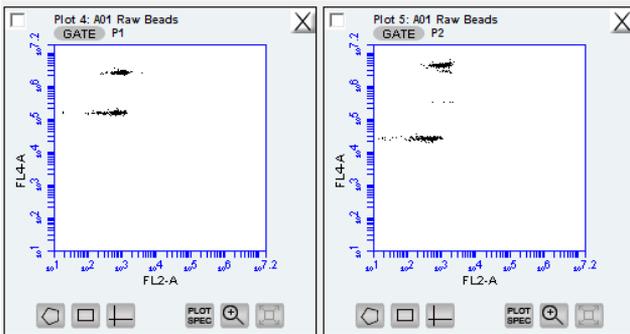
- 2.4 Vortex the vial of Raw Beads for 30 seconds to resuspend the beads. Transfer 400 μ L of the Raw Beads to a fresh FACS tube
- .
- 2.5 Set the fluidics flow rate to low/medium. Run the Raw Beads. The raw beads may appear small at the low left corner of the plot (Figure 21, left). Magnify FSC and SSC dot plot by clicking on the magnifying glass and draw a rectangular box around the populations, so that both bead populations are visible (Figure 21, right). Create two gates P1 (Beads A) and P2 (Beads B) respectively, for these two beads populations.

Figure 21.



- 2.6 Click GATE to change gating: show P1 in Plot 4 and P2 in Plot 5 (Figure 22).
Optional: the scale of Y-axis can be changed to bring the populations to the middle (e.g., change Y-axis to start at 10^3 instead of 10^1 . Figure not shown).

Figure 22.



- 2.7 Click File and save template as LEGENDplex template. The template is now ready.

3. Opening a template for data acquisition

To open an existing template, click File and then Open Workspace or Templates. Open the desired template from where the template is stored. After the template is opened, go to File and save the workspace as a new one for your new experiment. Your machine is now ready for data acquisition.