

Setup Procedure for Beckman Coulter Gallios™ Flow Cytometers

This part of the guide applies to Beckman Coulter flow cytometers using Gallios™ software.

For the Gallios™ Flow Cytometers, use the PE channel for reporter and the APC channel for beads classification. In general, there is no need for compensation between these channels if the machine is set up properly following the setup procedure described below.

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 process is not needed if you have run this experiment before and have access to a saved experiment protocol (The settings will be saved in the final step of this setup procedure and any settings saved can be imported to a new protocol. Please refer to Step 2 and Step 3.9 below).

1. Start up the Instrument

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

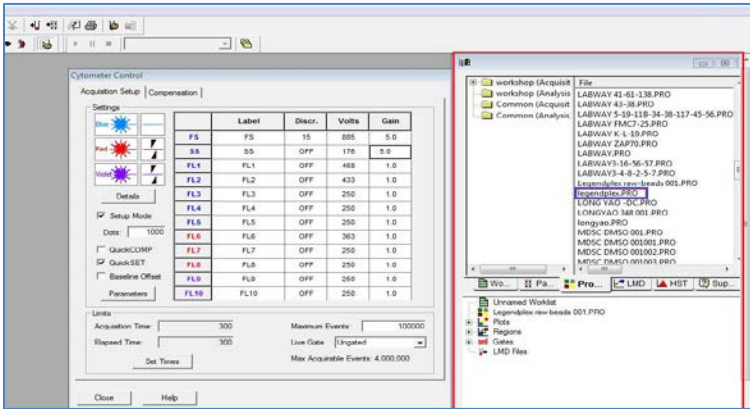
2. Obtain a Protocol for Data Acquisition

A protocol for Gallios™ is a workshop with density plots that allows the user to perform machine setup and data acquisition.

If a protocol is not yet available, create a new protocol by following the instructions below. After a protocol is created, save the file in Acquisition\Protocol\LEGENDplex. Do not change the name of the protocol folder.

If you have already created a protocol for the flow cytometer, open that protocol and proceed to Step 3. To open an existing protocol, drag the protocol to workshop. A list of protocols will pop up in the right box (Figure 19). Select the desired template from the list.

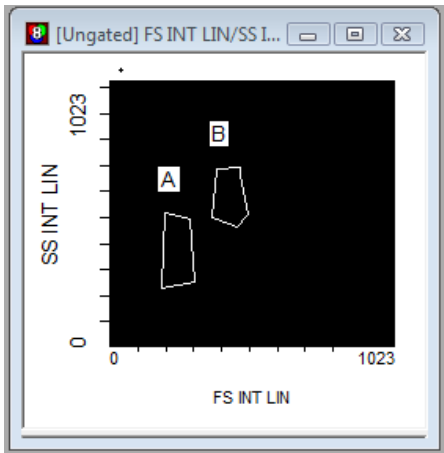
Figure 19.



To create a new protocol, follow the instructions below:

- 2.1 From the Gallios™ software, go to Files >> New >> New Protocol
- 2.2 In the workshop, create a 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.** Create two gates and label them A (Beads A) and B (Beads B) (Figure 20).

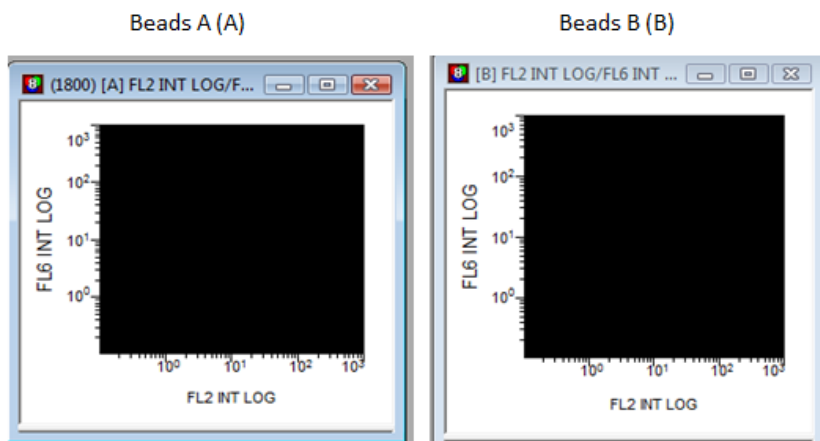
Figure 20.



- 2.3 Create two dot plots with PE for X-axis, APC for Y-axis (Figure 21), gated on A (left panel below) and B (right panel below), respectively. Create one dot plot with FITC for X-axis, APC for Y-axis, gated on Beads A and Beads B (graph not shown). **The plots should all be in**

log mode.

Figure 21.



- 2.4 Save the document as “LEGENDplex™ Protocol for Gallios software” in Acquisition\ Protocol or click Save Protocol, and proceed to the next step of setup.

3. Set up PMT Voltages

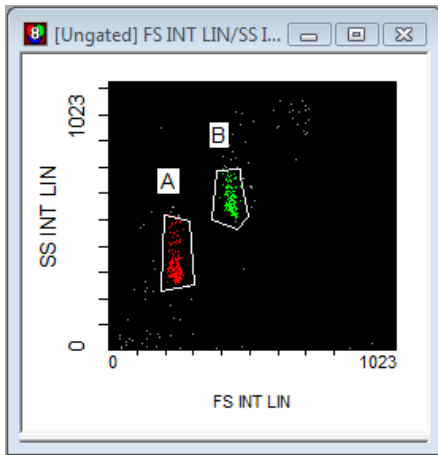
The Setup Beads 3: Raw Beads are used to set up the PMT voltage of the classification channel APC, reporter channel PE, and FITC channel. The Setup Beads 1: FITC Beads and 2: PE Beads are not needed for this setup because no compensation is required if the setup procedure described here is closely followed.

Follow the instructions below for setting up the PMT settings:

- 3.1 Vortex the vial of Raw Beads for 30 seconds to resuspend the beads.
- 3.2 Transfer 400 μ L of the Raw Beads to a fresh FACS tube.
- 3.3 Set the flow cytometer flow rate to low. Run the Raw Beads. Adjust the settings for FSC and SSC so that both bead populations are visible (Figure 22).

Pause and restart acquisition frequently during the setup procedure to refresh the beads populations after adjusting settings.

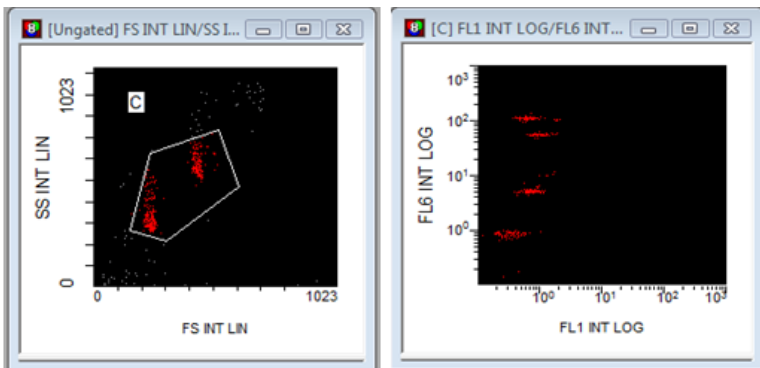
Figure 22.



- 3.4 Continue adjusting the settings so that Beads A and Beads B are well separated.
- 3.5 Move the gates (A and B) for Beads A and Beads B so that the smaller beads fall into Beads A gate and the larger beads fall into Beads B gate (Figure 22).
- 3.6 Adjust the FITC setting so that the FITC signal for the majority of beads is around 1×10^0 (Figure 23).

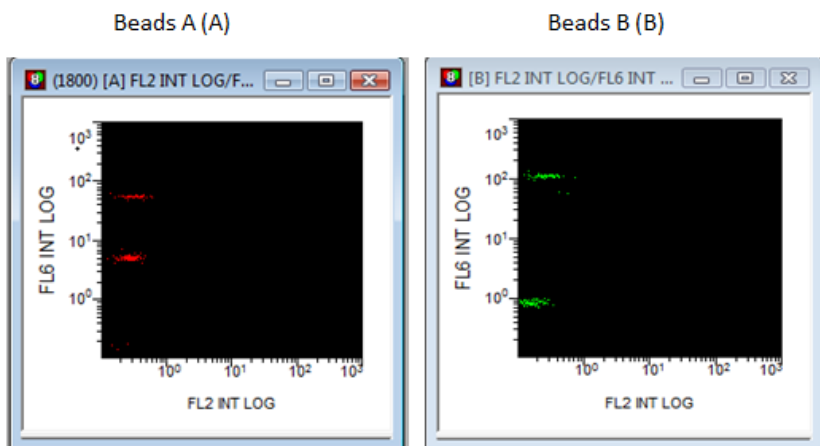
Figure 23.

Beads Beads A+ Beads B



- 3.7 Adjust the PE setting so that the PE signal for the majority of beads is around 1×10^0 (Figure 24).

Figure 24.



- 3.8 Adjust the APC settings so that the APC fluorescence intensities of all bead populations are between 1×10^0 and 2×10^2 (Figure 24).
- 3.9 Save the protocol again for future use.
- 3.10 The flow cytometer is now ready for sample analysis.