

## Setup Procedure for Acea NovoCyte® Flow Cytometer with Dual Lasers

For dual laser NovoCyte® flow cytometers, use the PE channel (BL2) for the reporter signal and the APC (RL1) channel for beads classification. Compensation between these channels is not required if the instrument is set up properly according to the procedure below.

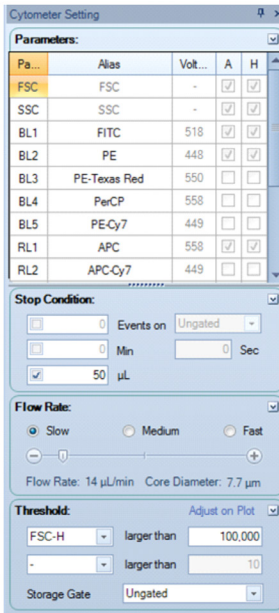
### 1. Start up the Instrument

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

### 2. Create Templates for Data Acquisition

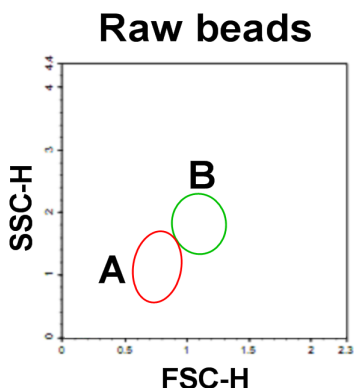
- 2.1. Select A (area) and H (height) for parameters FSC, SSC, FITC (BL1), PE (BL2), and APC (RL1) in the cytometer settings panel (Figure 1).

Figure 1.



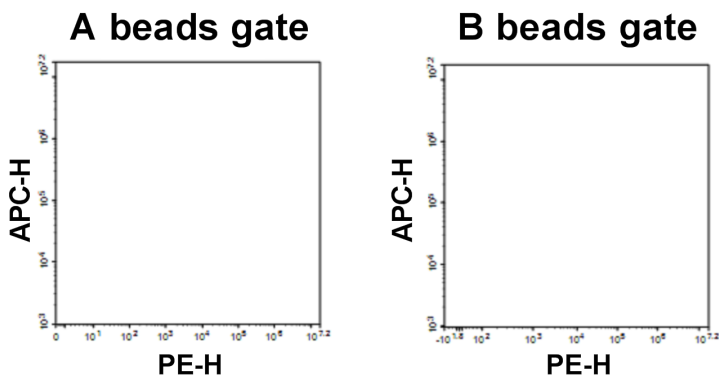
- 2.2. Create a density plot using FSC-H for the X-axis and SSC-H on the Y-axis. Create two gates and label them A and B (Figure 2).

Figure 2.



- 2.3. Create two density plots using PE-H for the X-axis and APC-H for the Y-axis, gated on A or B respectively. Set the axis mode of APC-H to log and PE-H to Bi-exponential by right clicking on the respective axis and selecting the appropriate mode (Figure 3).

Figure 3.



### 3. Setup the PMT Voltages

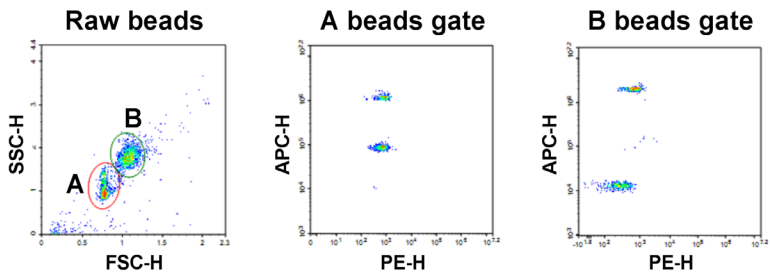
- 3.1. Vortex the vial of the Setup Beads 3: Raw Beads for 30 seconds to re-suspend the beads.
- 3.2. Transfer 400  $\mu$ L of the Raw Beads to a new FACS tube.

- 3.3. Acquire the Setup Beads 3: Raw Beads with the following stop conditions: 50  $\mu$ L, flow rate slow, and the FSC-H threshold set to 100,000.

**Note:** This stop condition is for the Setup Beads only. The acquiring volume is high because of the low density of the setup beads. When acquiring unknown experimental samples, the stop condition is set differently.

- 3.4. Move the A and B gates so that the smaller beads fall into the A gate and the larger beads fall into the B gate (Figure 4).

Figure 4.



- 3.5. Right click the PE axis on both the A and B beads gates and click **Reset Below Zero Value** to set the minimal PE axis automatically. Check APC signal of each beads population. APC-H signals of all beads should be in the range of 1000 to 10,000,000. Adjust the PMT voltage of APC channel if needed (Figure 4).
- 3.6. Vortex the vial of Setup Beads 2: PE Beads for 30 seconds to re-suspend the beads.
- 3.7. Transfer 400  $\mu$ L of the PE Beads to a new FACS tube.
- 3.8. Replace the Raw Beads tube from the cytometer with the PE Beads tube.
- 3.9. Using the same template and settings as above, acquire the PE beads. PE beads are only of small size, falling in the A beads gate. Verify that the median PE-H signal of the PE beads lies between 100,000 and 1,000,000. Adjust the PMT voltage of the PE channel in the cytometer settings panel if necessary.

- 3.10. As a general recommendation, run the highest standard (C7) prior to data acquisition to ensure that the APC and PE signals are not off scale (higher than 10,000,000). If necessary, adjust PMT voltage settings.
- 3.11. In the Cytometer Settings Panel, change the Stop Condition to 20  $\mu$ L and leave the number of events unchecked to collect the entire 20  $\mu$ L.
- 3.12. The flow cytometer is now ready for sample analysis.