

Ready, Set, Run!

The Collect tab is used to collect or import a sample, configure the run settings and set up plots and gates.

Collect a Sample

1. Start the C6 Flow Cytometer, open BD Accuri C6 Software and wait for the green “traffic light” message.
2. Place sample tube on the SIP.
3. Select a sample well in the grid.
4. Configure the run settings:
 - Set the run limits
 - Select fluidics settings
 - Set threshold (primary and optional secondary parameters)
5. Click **Run**.

Make a Plot

1. Click on the Histogram , Dot Plot or Density Plot button. The plot shows the selected sample.
2. Click the parameter name (e.g. FSC-A) to rename or change parameters from the drop-down menu.
3. Click the Plot Spec button to change linear to log, set an axis range or change parameters.

Note: Anything you do to your plot only changes the display of the data, not the data itself.

Tip! You can copy and paste the statistics tables into other applications and drag plots from C6 Software to other applications.

The screenshot displays the 'Collect' tab of the BD Accuri C6 software. It features a 12-well sample grid (A1-H12) with well A4 selected. Below the grid are 'Run Settings' (Run Unlimited, Run with Limits, 200,000 events, P3) and 'Fluidics' (Slow, Medium, Fast, Flow Rate 35 µl/min, Core Size 10 µm). A 'Threshold' section is also visible. The main area contains several plots: Plot 1 (A4 HPB CD3, CD4, CD45, CD8) with a gate, Plot 4 (A4 HPB CD3, CD4, CD45, CD8) with a gate, Plot 5 (A4 HPB CD3, CD4, CD45, CD8) with a gate, Plot 10 (A4 HPB CD3, CD4, CD45, CD8) with a gate, Plot 7 (A4 HPB CD3, CD4, CD45, CD8) with a gate, and Plot 8 (A4 HPB CD3, CD4, CD45, CD8) with a gate. Below the plots are statistics tables for each plot.

Plot 1: A4 HPB CD3, CD4, CD45, CD8	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median
All	229,303	0	100.00%	100.00%	418,636.29	110,634.37	71.60%	164.34%	292
P3	104,355	0	45.51%	45.51%	252,480.06	27,325.01	18.60%	37.45%	246

Plot 4: A4 HPB CD3, CD4, CD45, CD8	Count	Volume (µL)	% of This Plot	% of All	Mean CD45 PE-CY7-A	Mean SSC-A	CV CD45 PE-CY7-A	CV
All	229,303	0	100.00%	100.00%	21,257.10	110,634.37	97.17%	11
P2	102,946	0	44.90%	44.90%	38,804.74	28,392.16	26.74%	29
P4	76,792	0	33.49%	33.49%	5,163.14	218,886.76	36.44%	1
P5	8,059	0	3.51%	3.51%	19,973.85	121,487.41	25.48%	1

Plot 5: A4 HPB CD3, CD4, CD45, CD8	Count	Volume (µL)	% of This Plot	% of All	Mean CD3 FITC-A	CV CD3 FITC-A	Median CD3 FITC-A
This Plot	102,946	0	100.00%	44.90%	23,511.96	63.89%	26,241.5
M1 (5,519.0 / 109,310.0)	79,338	0	77.07%	34.60%	30,330.70	30.96%	29,634.0

Make a Gate

1. Click on a **Marker** button to draw a region to use as a gate.
2. Click the gate **GATE** button on the plot to which the gate should be applied.
3. In the Gating dialog, choose which regions to Include , Exclude or Intersect and then click **Apply**.

Apply Color Compensation

1. Click the **Set Color Compensation** button.
2. In the Color Compensation dialog, select the parameters you want to compensate, and then enter the compensation percentages.
3. Apply settings to current or all samples.
4. Click **Apply & Close**.

Zooming In or Out on a Plot

- To zoom in on a plot, click the **Zoom In** button and encompass the region of interest using the cursor.
- Zoom can be used multiple times on the same plot.
- To zoom out on a plot, click the **Zoom Out** button to return to the previous view.