Job Aid

BD FACSDiscover™ A8 Cell Analyzer: Setting up and recording single-stained controls using the manual tube port

This job aid contains instructions for how to set up and record single-stained controls for imaging and high-speed experiments in BD FACSChorus™ Software. For additional information, see the *BD FACSDiscover™ A8 Cell Analyzer with BD CellView™ and BD SpectralFX™ Technology user's guide*.

Start up system → Set up experiment → **Establish settings** → Record and analyze → Manage data → Shut down system

Before you begin

- Start up the system and run a daily or extended fluidics startup procedure.
- For an imaging experiment, create and design an experiment, adjust your scatter and spectral gains, and set the Region of Analysis (ROA) for your sample. Ensure that the ROA has been set up on the Adjust Gains or View Data page for the specific particles used in your fluorochrome controls.
- For a high-speed experiment, create and design an experiment, and adjust your scatter and spectral gains for your sample.

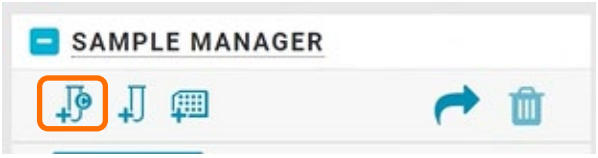
Working with the Set Up Single-Stain Controls tab

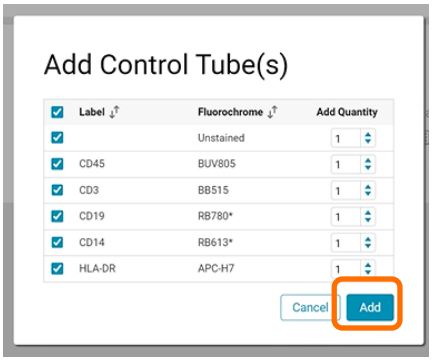
Adding the control tubes

Use the Set Up Single-Stain Controls page to set up the Sample Manager and record the control tubes individually for your experiment.

1. Click the **Set Up Single-Stain Controls** tab.
2. In the Sample Manager panel, click the **Add Control Tube(s)** icon.

   One unstained control is automatically added.
3. Leave all the checkboxes selected if you want to add all the controls and if needed, change the quantity.
4. Click **Add**.



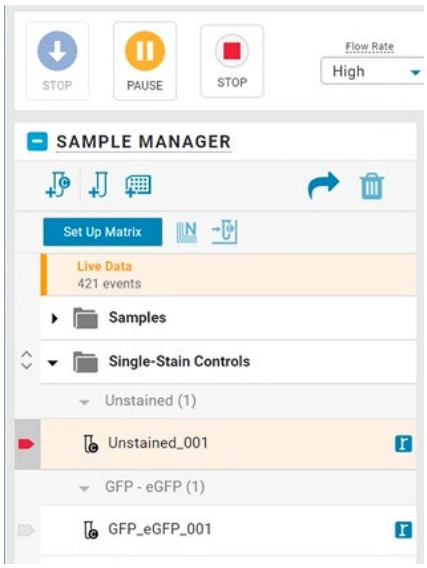


Adding and acquiring the control tubes

1. Click to select the control tube in the Sample Manager panel.
2. Load a control tube on the manual tube port.

   NOTE Control tubes can be acquired in any order.

   NOTE For an imaging experiment, before acquiring your single-color controls, make sure the ROA has been set up for the specific particle in your sample using the Adjust Gains page.
3. (Optional) Edit the name for the unrecorded control name.
4. Click **Acquire**.



Adjusting the settings

1. Adjust the plot zoom, scatter gains, threshold, plot scaling, and gates as necessary to encompass cells of interest.

   a. Zoom in on a plot by clicking the plot, then rolling the mouse scroll wheel upwards. Roll downwards to zoom out.

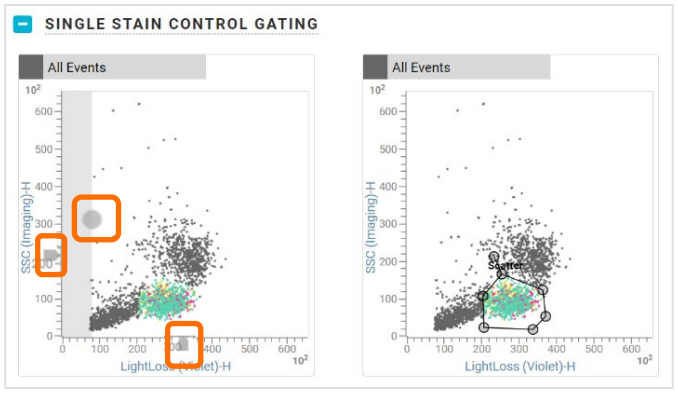
   b. Adjust the scatter gains, if needed. Hover over the scatter plot's axes then drag the gain sliders along the axes.

      NOTE For imaging experiments, if the SSC-Imaging gain is changed, then the ROA must be reset for the control particles before recording data.

   c. Adjust the threshold by hovering over the gray portion of the first default plot and then dragging the threshold marker (gray dot) horizontally.

   d. Adjust the plot scaling by using the biexponential scale slider.

   e. Adjust the gates by dragging the vertices to encompass populations.



2. Verify that no channels are saturated on the spectral plot.

   Saturated channels are indicated by a colored box with the (!) icon, which displays at the bottom of the affected channel.

   Lower the gains of any saturated channel.

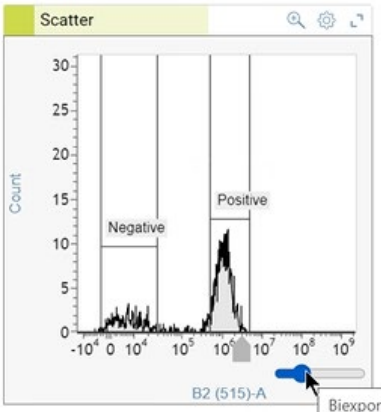
   a. Click the down arrow on the top left corner of the spectral plot panel.

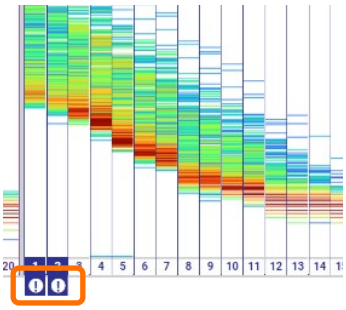
   b. Adjust the spectral plot gains under Gain Value by using one or more of the following techniques:

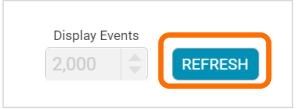
      - Enter a new value for the channel, then press **Enter**.
      - Select a channel, then scroll using your mouse scroll wheel.
      - Click the + and – buttons to adjust gains for all channels in that laser.

      Click **Reset All** to set the gains of all channels to the default settings.

   TIP After adjusting the gain, click **Refresh** in the dashboard to visualize the change.

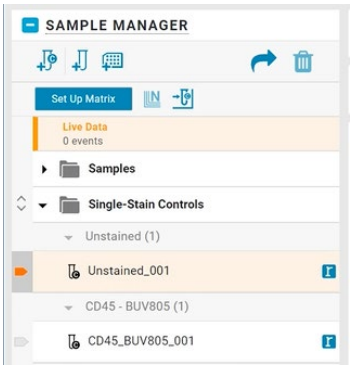






Recording controls using the manual tube port

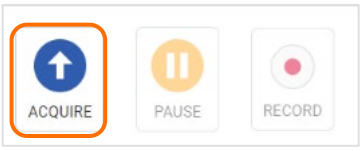
1. Click to select a control tube in the Sample Manager panel.



2. Verify that the appropriate control tube is on the manual tube port.

3. (Optional) Change the number of events to record in the FCS Stopping Criteria field. **NOTE** The default is 10,000.

4. Click **Acquire** in the dashboard.

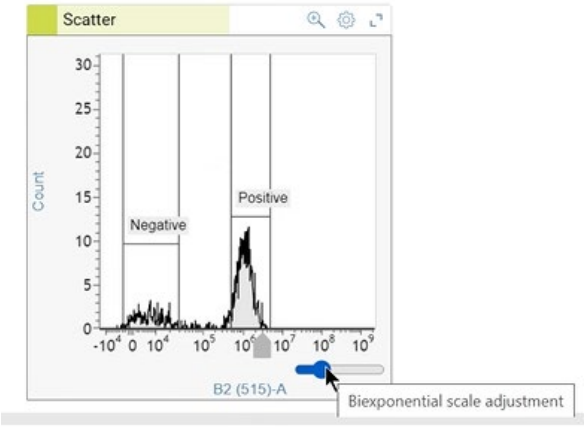


5. Adjust the negative and positive interval gates on the histogram to encompass at least 100 events.

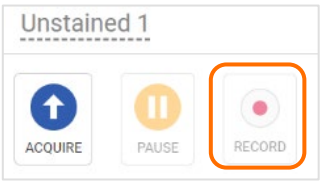
   NOTE Unstained control tubes only have a negative gate.

   NOTE Gates can be adjusted before, during, or after recording.

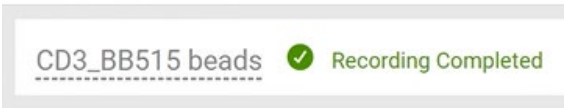
   NOTE As an alternative for the negative population in your single-stained controls, you can select a previously recorded unstained tube as your negative control.



6. Click **Record**.

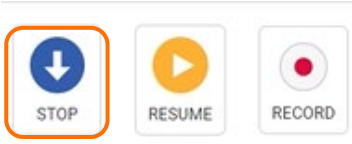


   Once the histogram gates encompass at least 100 events, the control tube recording is confirmed automatically.



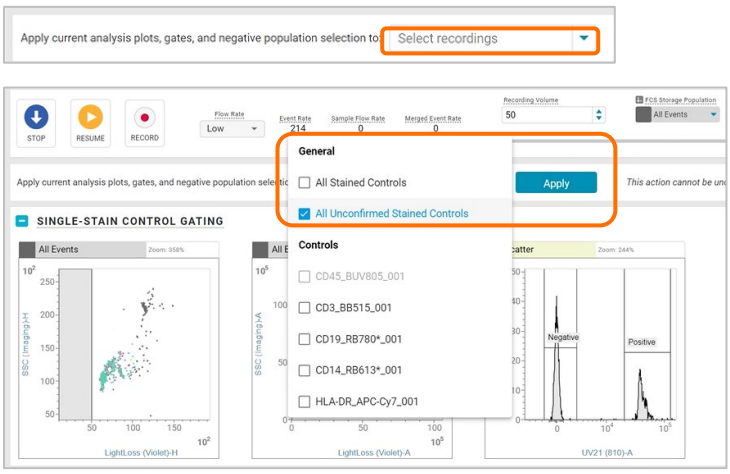
7. Verify the interval gates are set appropriately.

8. Click **Stop**.

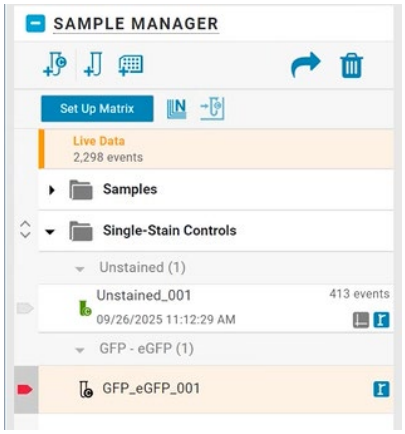


Recording controls using the manual tube port, continued

TIP To apply the plots, gates and negative populations to the remaining applicable control tubes, click the **Select recordings** dropdown menu by selecting **All Unconfirmed Stained Controls.** Click **Apply.**



9. Click to select the next control tube in the Sample Manager panel.



10. Load the next control tube on the manual tube port and repeat steps 1–8 for the remaining controls.

CAUTION Wait for the system backflush to complete. The status indicator light will change from blue to green when complete. Loading the tube early will cause the sample to be aspirated from the tube.

TIP For imaging experiments, if single-stain controls are not all the same particle type (for example, some control tubes contain beads and some contain cells), we recommend first verifying ROA for one particle type, and recording all tubes containing that particle type. Then, verify ROA for the second particle type and record all tubes containing that particle type.

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