Job Aid

BD FACSDiscover™ S8 Cell Sorter: Setting up and recording single-stained controls

This job aid contains instructions for how to set up and record single-stained controls for your experiment in BD FACSChorus™ Software. For additional information, see the *BD FACSDiscover™ S8 Cell Sorter with BD CellView™ and BD SpectralFX™ Technology user's guide*.

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

Before you begin

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

Working with the Set Up Single-Stain Controls tab

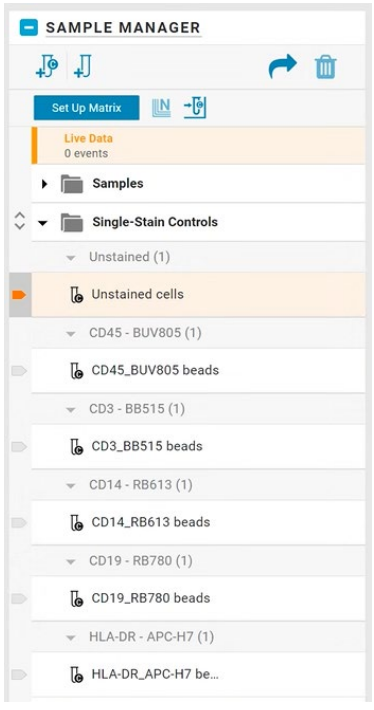
Setting up single-stained controls

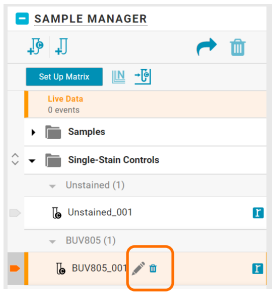
1. Click the **Set Up Single-Stain Controls** tab.

   The Sample Manager is automatically populated with control tubes based on the fluorochromes selected in the Fluorochrome Selection panel.

   NOTE Autofluorescence can be added later using any unstained control.

   TIP To change the name of the tube, hover over the tube name and use the pencil icon.





2. Load a control tube on the sample loading port.

   NOTE Control tubes can be acquired in any order.

   NOTE Data does not to be recorded in the unstained tube in order to calculate unmixing, however only unstained tubes can be used for autofluorescence.

3. Select the appropriate tube from the list and click **Load** in the dashboard.

Adjusting settings

1. Adjust the plot zoom, scatter gains, threshold, plot scaling, and gates 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.

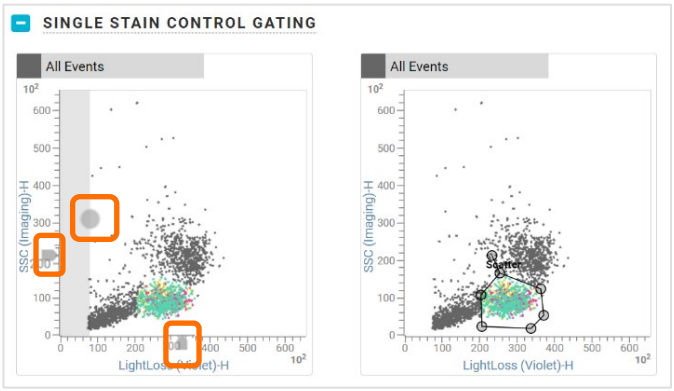
   b. Adjust the scatter gains, if needed. Hover over the scatter plots axes then drag the gain sliders along the axes.

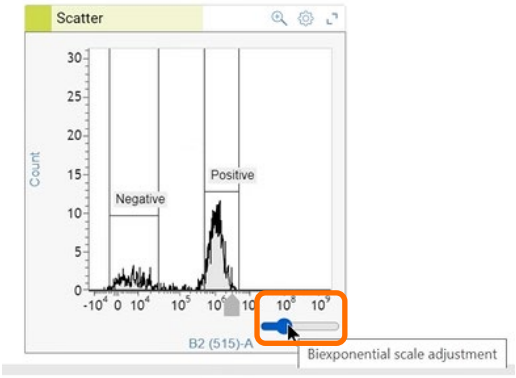
      NOTE If the SSC-imaging gain is changed, the ROA for the control particles must be reset before recording data.

   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 clicking the biexponential scale adjustment 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.

   NOTE Reducing the spectral gain will not reduce the imaging gains for the same color. You must reduce the imaging gains separately.

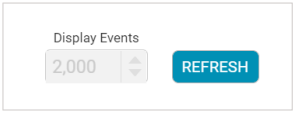
   a. Click the down arrow on the top left corner of the spectral plot panel.

   b. Adjust the spectral plot gains under Gain Value:

      - 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

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

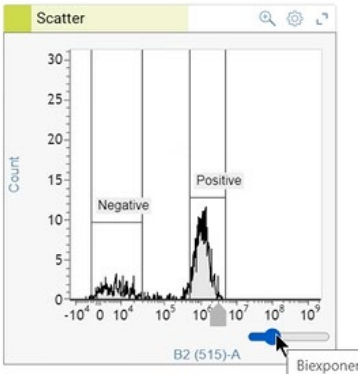
2. Click **Load** in the dashboard.

3. Adjust the scatter and histogram (interval) gates to encompass positive and negative populations of interest.

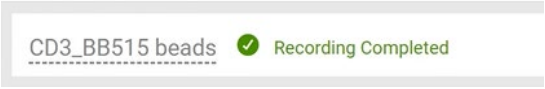
   NOTE As an alternative for the negative population in your single-stained controls, you can select a previously recorded unstained tube as your negative control.

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

   TIP In order to add multiple autofluorescence controls, gate the population(s)in the unstained tube.



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



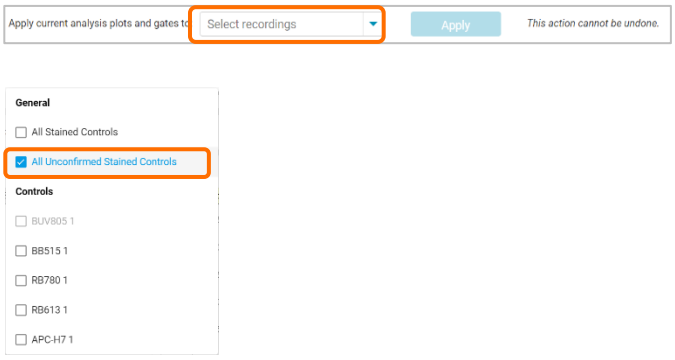
4. Load the next control tube on the sample loading port.

5. Select the appropriate tube from the list and click **Load** in the dashboard.

6. Repeat steps 1 through 5 for all the remaining controls.

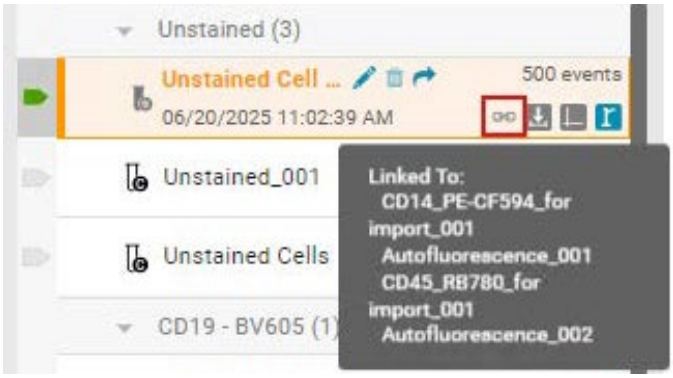
   TIP If single-stain controls are not all the same particle type (for example, some control tubes contain beads and some contain cells), we recommend first verifying ROA for one particle type, and recording all tubes containing that particle type. Then verify ROA for the second particle type and record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

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



Recording controls, continued

NOTE If an available recorded unstained control is used as a negative control with any of the fluorochrome single-stain controls, a Link icon displays next to the unstained control in the Sample Manager panel. Hover over the **Link** icon to display the fluorochrome single-stain controls linked to the unstained tube.



BD Life Sciences, Milpitas, California, 95035, USA

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