

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 FACSCorus™ Software. For additional information, see the *BD FACSDiscover™ S8 Cell Sorter with BD CellView™ and BD SpectralFX™ Technology user's guide*.



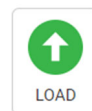
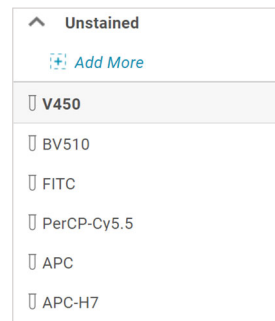
Before you begin

- 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 page is automatically populated with control tubes based on the fluorochromes selected in the Select Your Dyes panel, including Autofluorescence.
2. (Optional) Click **+Add More** to add separate Unstained control tube(s).
Click the edit (✎) icon to rename the Unstained control(s), if needed.
3. Load a control tube on the sample loading port.
4. Select the appropriate tube from the list and click **Load** in the dashboard.



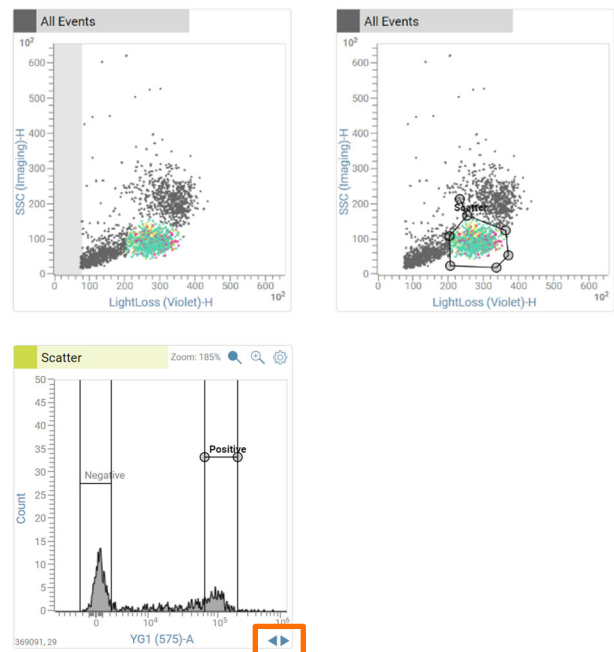
Adjusting settings

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, then the ROA must be reset for the control particles before recording data.

 - c. Adjust the threshold by hovering over the y-axis of the first default plot and then dragging the threshold marker horizontally.
 - d. Adjust the plot scaling by clicking the biexponential scale adjustment arrows.
 - e. Adjust the gates by dragging the vertices to encompass populations.

SINGLE STAIN CONTROL GATING



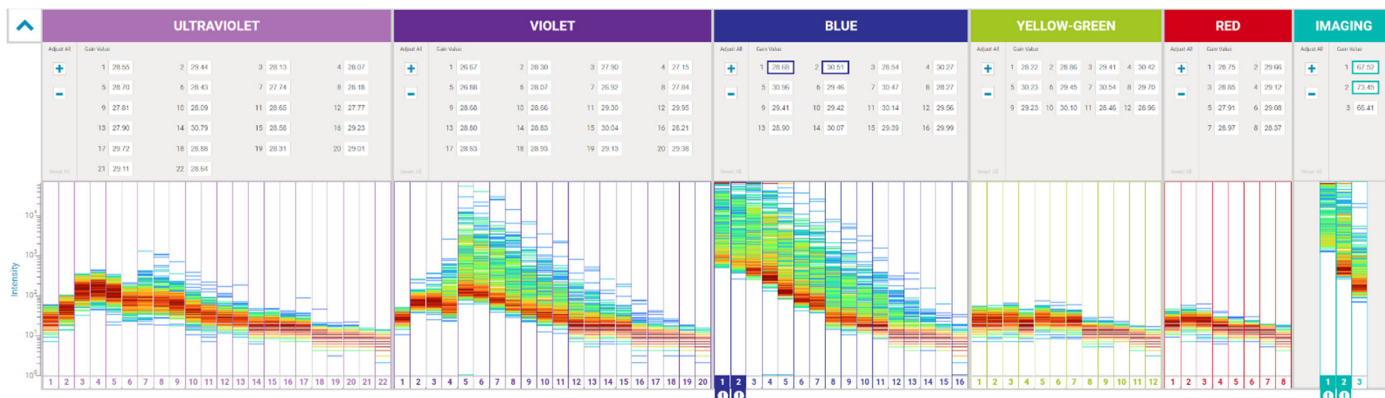
2. Verify that no channels are saturated on the spectral plot.

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.

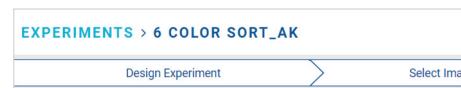
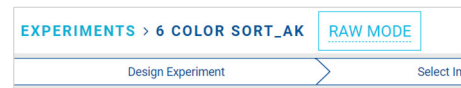
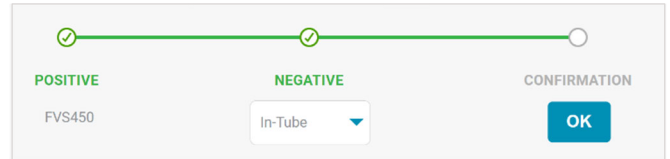
- a. Click the down arrow on the top left corner of the spectral plot panel.
- b. Adjust the spectral plot gains under Gain Value:
 - Enter a new value for the channel, then press Enter.
 - Select a channel, then scroll using your mouse scroll wheel.
 - Click the + and - buttons to adjust gains for all channels in that laser.

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



Recording controls

1. Click **Record** in the dashboard.
2. Adjust the scatter and histogram (interval) gates to encompass positive and negative populations of interest.
If needed, select a previously recorded unstained tube as your negative control.
Gates can be adjusted at any time before, during, or after recording.
3. Click **OK** under Confirmation.
NOTE This step is not necessary for the Unstained control tubes.
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.
7. If controls are comprised of multiple particle types, verify the ROA for each particle type on the View Data page.
After the last control tube has been confirmed, verify that the Raw Mode indicator next to the experiment name is not displayed.



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