

# 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 FACSCorus™ Software. For additional information, see the *BD FACSDiscover™ A8 Cell Analyzer with BD CellView™ and BD SpectralFX™ Technology user's guide*.



### Before you begin

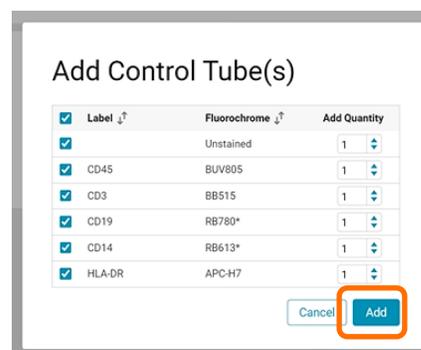
- Start up the system and run a daily or extended fluidics startup procedure.
- For an imaging experiment, create and design an experiment, adjust your scatter and spectral gains, and set the Region of Analysis (ROA) for your sample. Ensure that the ROA has been set up on the Adjust Gains or View Data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



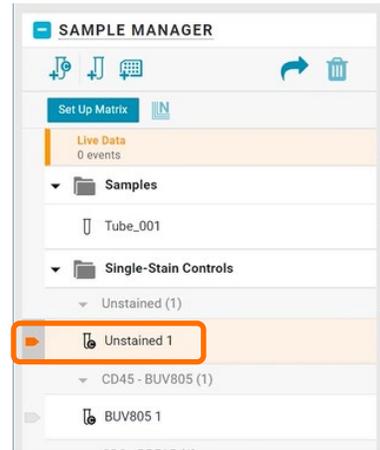
## Adding and acquiring the control tubes

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 plots 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.

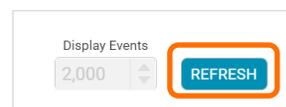
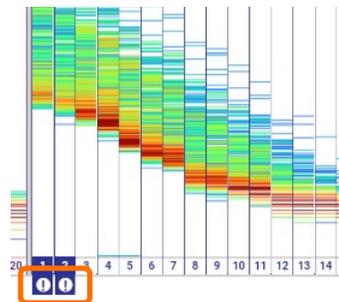
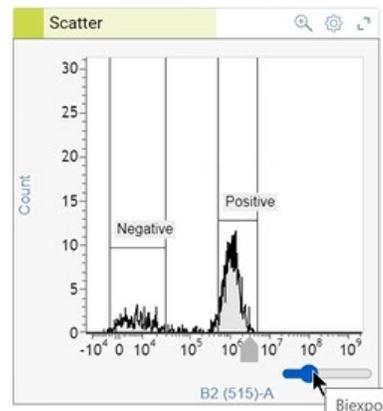
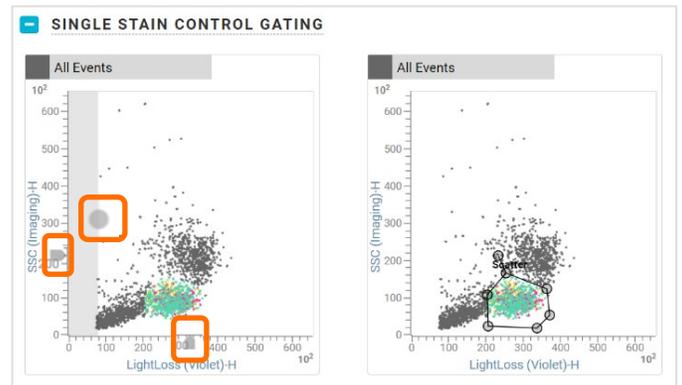
Saturated channels are indicated by a colored box with the (!) icon, which displays at the bottom of the affected channel.

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. Verify that the appropriate control tube is on the manual tube port.
2. (Optional) Change the number of events to record in the FCS Stopping Criteria field. **NOTE** The default is 10,000.
3. Click **Acquire** in the dashboard.
4. 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.

5. Click **Record**.
6. Verify the gates are set appropriately, then click **OK** to confirm the control.

**NOTE** You do not need to confirm the Unstained control tubes.

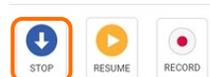
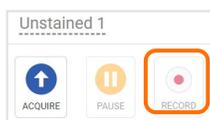
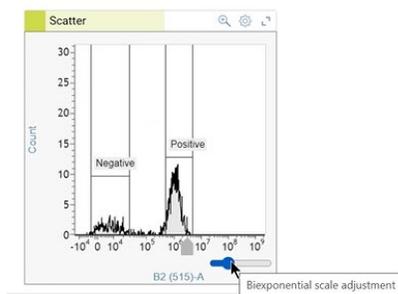
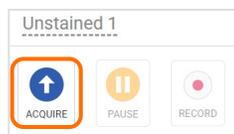
7. Click **Stop**.

**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**.

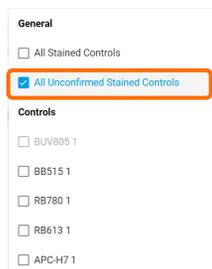
8. Click to select the next control tube in the Sample Manager panel.

9. Load the next control tube on the manual tube port and repeat steps 1–9 for the remaining controls.

**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.



Apply current analysis plots and gates to Select recordings Apply This action cannot be undone.



## Set up Matrix

Use the Set Up Matrix icon in the Sample Manager panel to perform spectral unmixing of the recorded fluorescence data which generates the spectral unmixing matrix. After the spectral unmixing matrix is generated, the Raw Mode icon no longer displays next to the experiment name. The spectrally unmixed data can be viewed on the View Data page.

**NOTE** The spectral unmixing matrix in a template experiment or a duplicated experiment carries over from the original experiment, but it is recommended that you re-run the controls and the spectral unmixing matrix must be set up again in the new experiment.

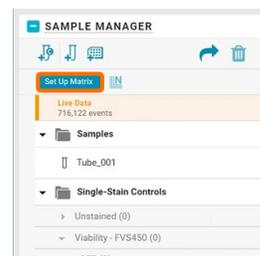
After you have confirmed at least one recording for all the controls in your experiment, proceed to set up the spectral unmixing matrix.

**NOTE** If the spectral unmixing process is not completed or is not successful, the Raw Mode icon will continue to display at the top of the Experiment workflow next to the experiment name, and no spectrally unmixed data can be viewed on the View Data page.

**NOTE** The Set Up Matrix icon is activated only after the system determines that you have at least one confirmed recording for each control.

**NOTE** Autofluorescence controls can be assigned only in the Set Up Matrix window.

1. Click **Set Up Matrix** in the Sample Manager.
2. Select the recording to use for each fluorescent single-stain control.
3. (Optional) Click **Add Autofluorescence Control** on the bottom left and select the unstained control to use for unmixing autofluorescence. This will add an additional plot to the preview for each included autofluorescence control.
4. Click **Generate Spectral Matrix**.
5. Confirm that a matrix was generated with a timestamp.
6. Close the Set Up Spectral Matrix page by clicking the **X** in the top right of the window.



Single-Stain Control (5)	Recording to Use	Negative
<input checked="" type="checkbox"/> CD45 - BUVR805	CD45_BUVR805_001	In-Tube
<input checked="" type="checkbox"/> CD3 - BB515	CD3_BB515_001	In-Tube
<input checked="" type="checkbox"/> CD19 - RB780*	CD19_RB780*_002	In-Tube
<input checked="" type="checkbox"/> CD14 - RB613*	CD14_RB613*_002	In-Tube
<input checked="" type="checkbox"/> HLA-DR - APC-Cy7	HLA-DR_APC-Cy7_002	In-Tube

+ Add Autofluorescence to use for Spectral Matrix

Generate Spectral Matrix

Revert to Raw Mode

Successfully generated Latest Matrix 03/20/2025 10:12:58 am

Generate Spectral Matrix

Revert to Raw Mode



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