Job Aid

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

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 with the loader. For additional information, see the *BD FACSDiscover™ A8 Cell Analyzerr 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 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 Sample Manager

The Sample Manager panel on the Single-Stain Controls page allows you to view, add, edit, or delete a multi-well plate or a tube rack. After adding a carrier type from the Sample Manager panel, use it to acquire and record data for multiple single-stained controls or unstained controls, or both, on a multi-well plate or in multiple tubes on a tube rack.

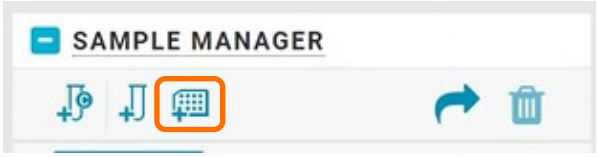
Adding the single-stained controls to a multi-well plate or tube rack

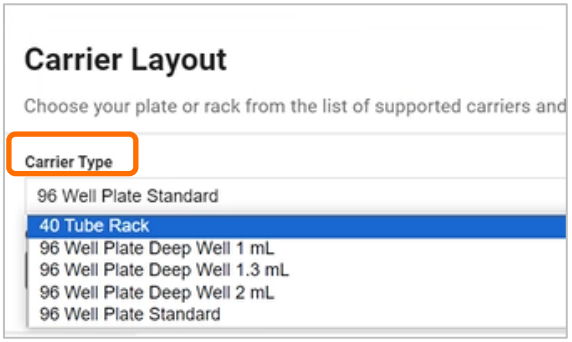
1. Click the **Set Up Single-Stain Controls** tab.

2. In the Sample Manager panel, click the **Add plate/tube rack** icon.

   NOTE Single-stained controls may be run in a tube rack or multi-well plate.

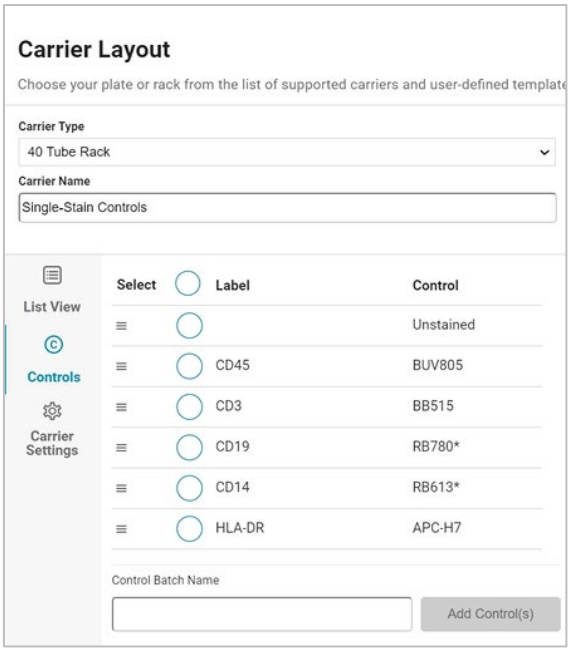
3. Select the Carrier Type.





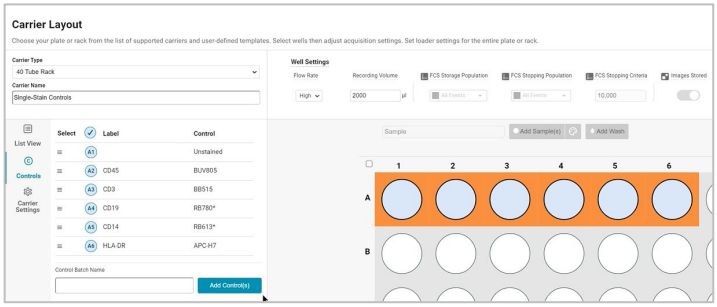
Adding the single-stained controls to a multi-well plate or tube rack, continued

NOTE By default, all controls are selected, which is indicated by the blue circles. If you are using an internal negative control, click the Unstained circle to deselect, and it will turn gray.



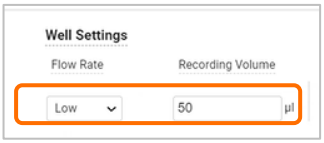
Setting up the rack or plate for the controls

1. Click well location **A1** to select and assign the controls simultaneously to the plate or rack.



NOTE If the labels in the software do not match the order in your carrier, you can drag and drop the controls to match the order of your carrier.

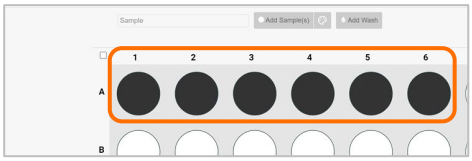
TIP From the Well Settings panel, set the Flow Rate to Low and decrease the Recording Volume to preserve the sample.



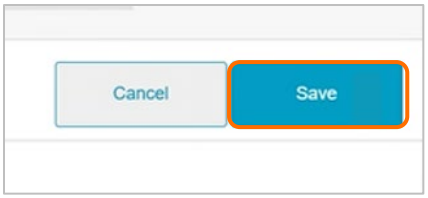
NOTE The other Well Settings are not available for use when setting up the controls.

2. Click **Add Controls**. The wells turn black once the controls are assigned.

TIP You can add a control batch name to help distinguish different types of controls.

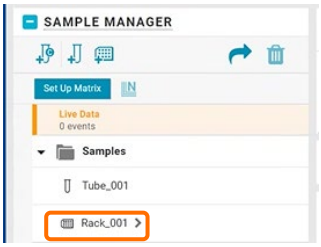


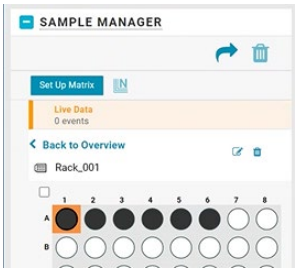
3. Click **Save** to save the Controls Carrier Layout settings.



Recording the controls

1. Click **Rack_001** or **Plate_001** to expand the view.

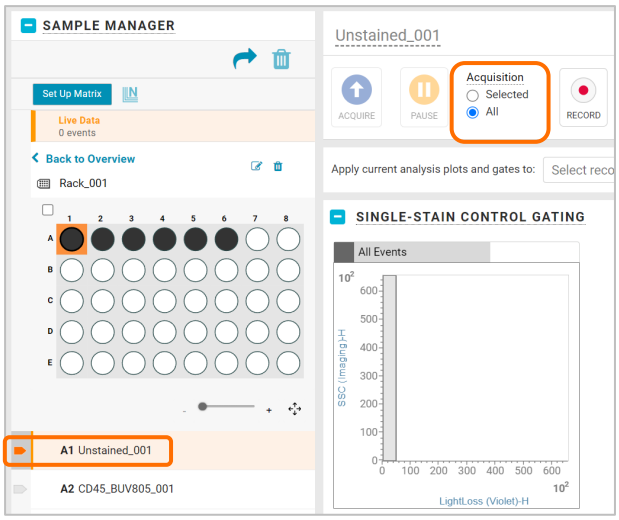




2. Select **All** for the Acquisition type.

   This option will run the controls sequentially.

3. Set your FCS recording, storage, and stopping criteria as needed.



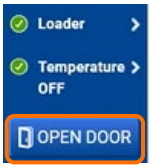
4. Load the plate or rack.

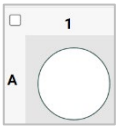
   a. Install a DI water tube on the SIT.

   b. Click **OPEN DOOR.**

   c. Place the plate or rack in the loader nest in the correct orientation with A1 in the upper-left corner.

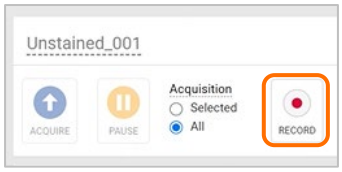
   d. Click **CLOSE DOOR.**

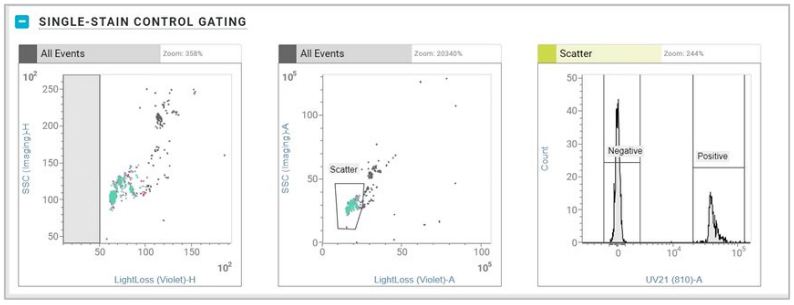




5. Click **Record** to record all of the controls.

   NOTE When one or more well or tube locations are selected, you cannot acquire but can only record.





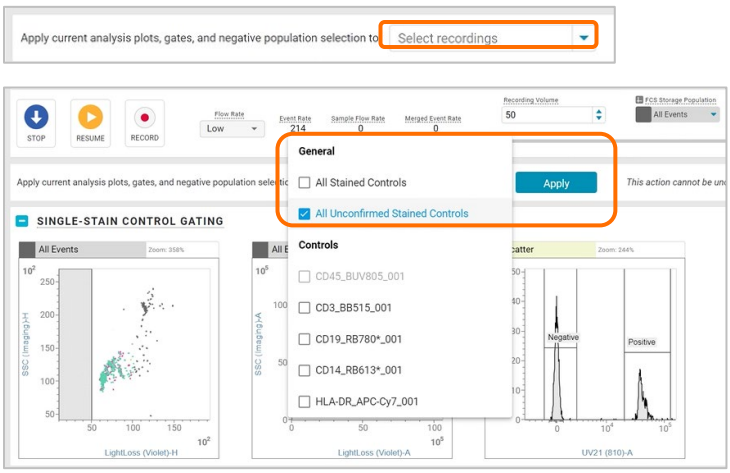
6. Adjust the plot scaling and the interval gate positions while recording, or wait until the last control is recorded.

7. Click **OK** to confirm each control.

   NOTE This step is not necessary for the Unstained control tubes.

Recording the controls, continued

TIP After the first single-stained control has been confirmed, you can use the **Select recordings** dropdown menu to apply the plots, gates and negative populations to the remaining control tubes.



8. After confirming all controls, click **OPEN DOOR** and remove the plate or rack, then click **CLOSE DOOR**.

Performing spectral unmixing

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.

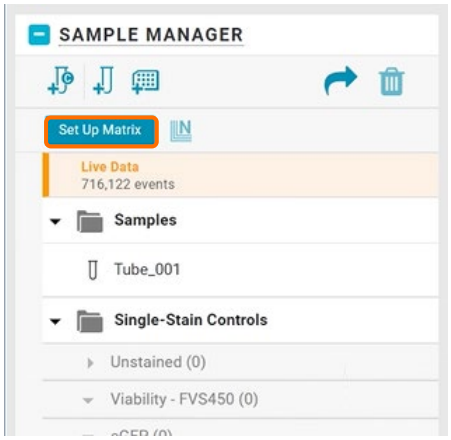
NOTE The spectral unmixing matrix in a template experiment or a duplicated experiment carries over from the original experiment, but it is recommended that you re-run the controls and the spectral unmixing matrix must be set up again in the new experiment.

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.

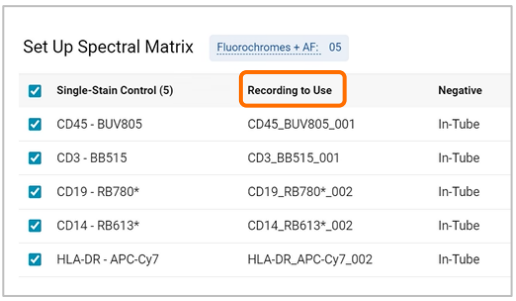
NOTE Autofluorescence controls can be assigned only in the Set Up Matrix window.

1. Click **Set Up Matrix** in the Sample Manager.

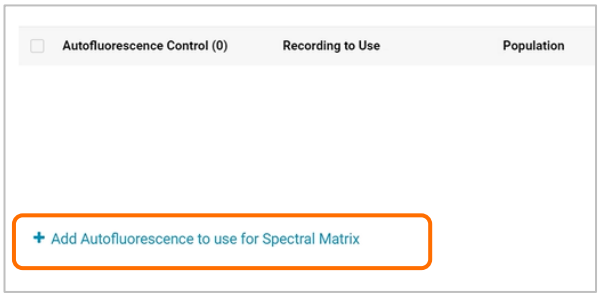


Performing spectral unmixing, continued

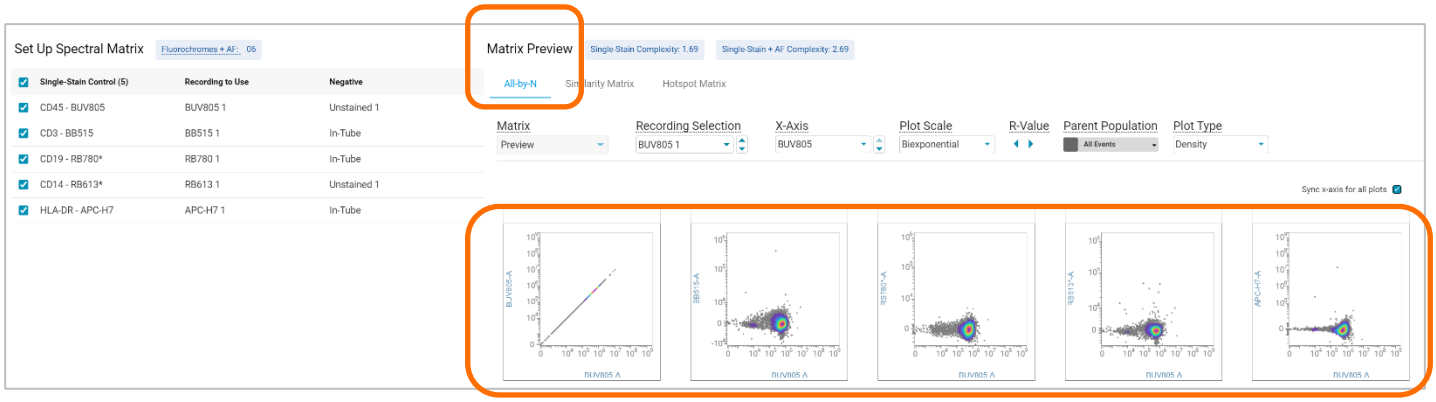
2. Select the recording to use for each fluorescent single-stain control.

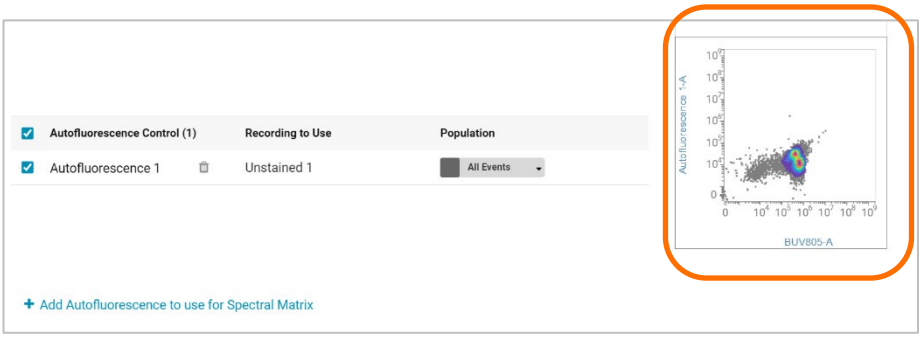


3. (Optional) Click **Add Autofluorescence Control** in the bottom left and select the unstained control to use for unmixing autofluorescence.



4. Click **Generate Spectral Matrix.**





The Matrix Preview displays the single-stained controls as All-by-N plots.

An additional Autofluorescence plot displays for each included autofluorescence control.

Performing spectral unmixing continued

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.

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