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 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 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

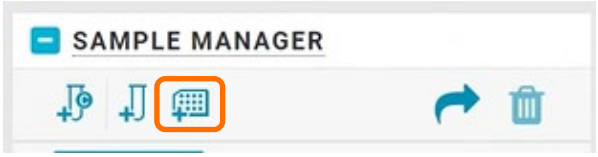
1. Click the **Set Up Single-Stain Controls** tab.

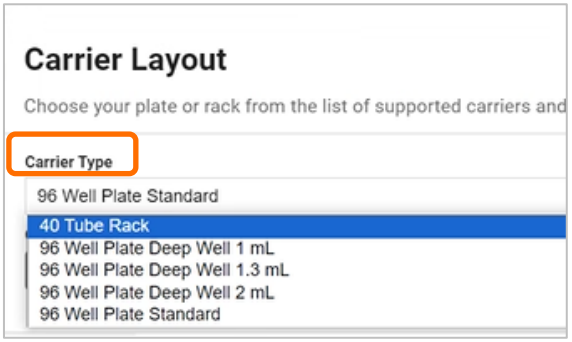
2. In the Sample Manager panel, click the **Add plate/tube rack** icon.

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

3. Select the Carrier Type.

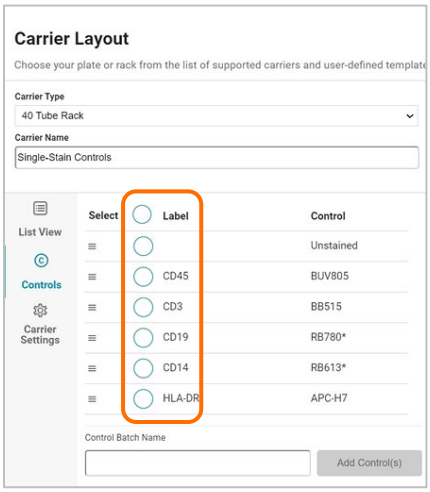
   NOTE Make sure to select the correct plate type. A 96-well plate is the default. If the wrong plate is selected, the sample will not be loaded properly.





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

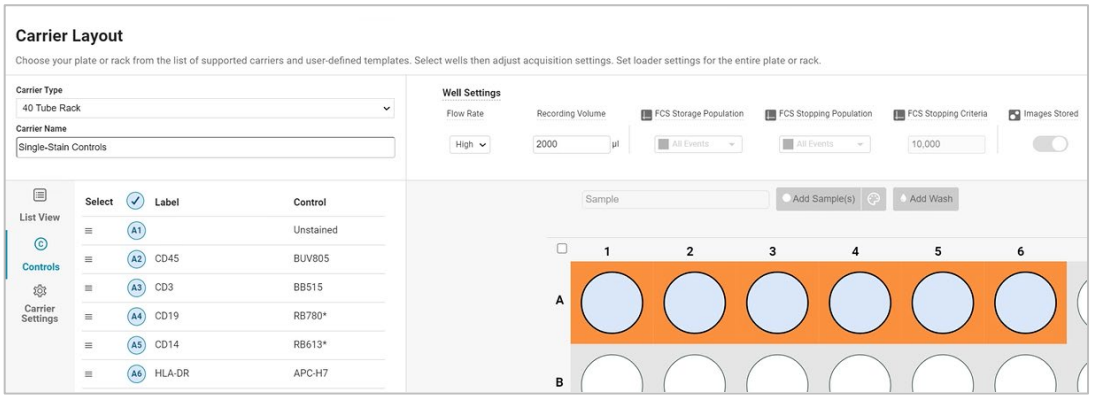
NOTE By default, all controls are selected, which is indicated by the blue circles. If you are using an internal negative control, click the Unstained circle to deselect, and it will turn gray.



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.

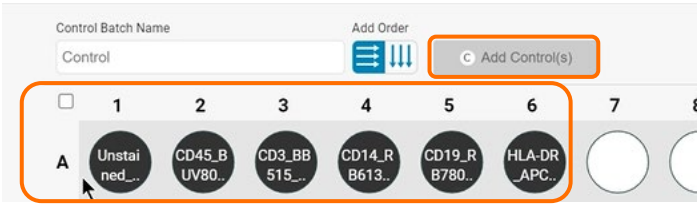


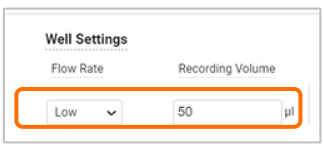
2. Click **Add Controls**. Once the controls are assigned, the wells turn black and the control labels appear.

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

   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.





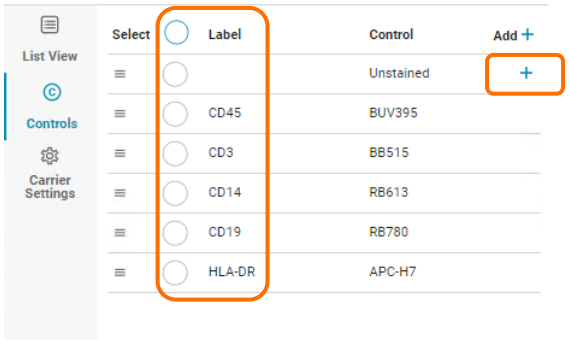
3. Click **Save** to save the Controls Carrier Layout settings.

Setting up the rack or plate for the controls, continued

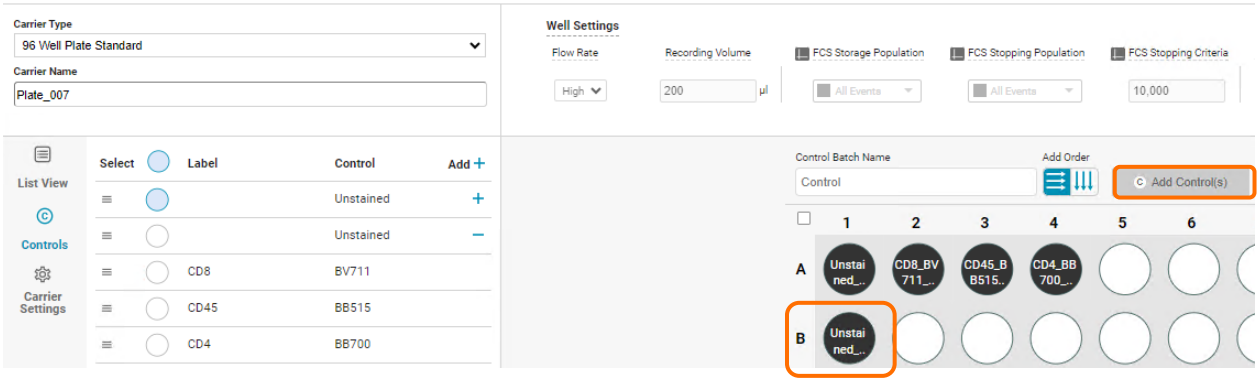
4. (Optional) Add additional unstained controls to the plate layout.

   a. Clear the label option so that none of the controls are selected (indicated by gray circles).

   b. Click the Add **(+)** for the unstained control.



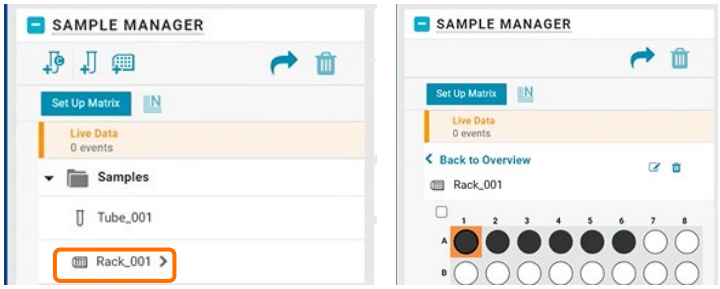
   c. Click the well location(s) for the added unstained control(s), then click **Add Controls**.



   d. Click **Save.**

Recording the controls

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

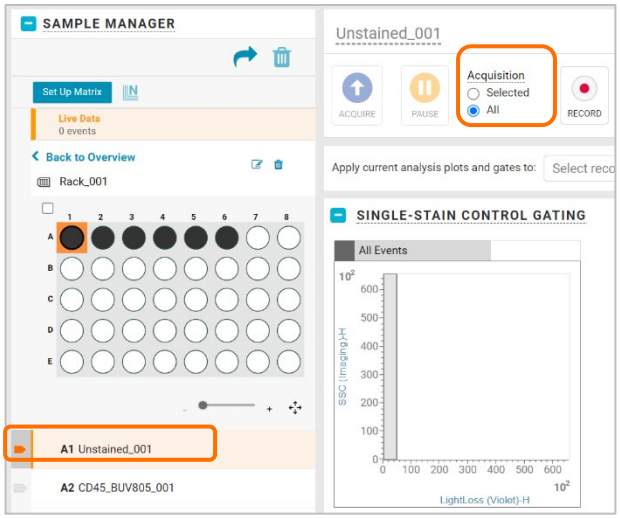


Recording the controls, continued

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.

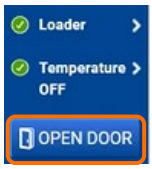
   a. Install a DI water tube on the SIT.

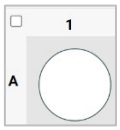
   b. Click **OPEN DOOR.**

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

   NOTE Make sure to orient the plate to match the sample manager.

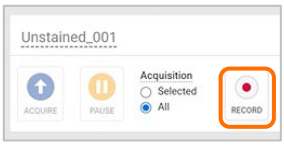
   d. Click **CLOSE DOOR.**





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.

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

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.

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

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