

## Job Aid

# BD FACSDiscover™ Family: Performing spectral unmixing

This job aid contains instructions for how to perform spectral unmixing for your experiment in BD FACSDiscover™ Software. For additional information, see the BD FACSDiscover™ A8 Cell Analyzer or BD FACSDiscover™ S8 Cell Sorter user's guides.



### 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 if imaging, adjust the Region of Analysis (ROA) for your sample.
- Record single stain controls.

### Working with the Set Up Matrix

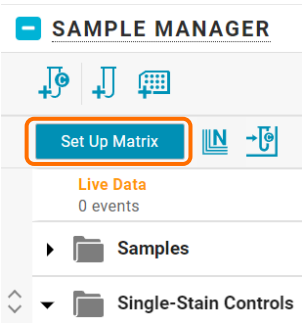
After successfully recording and confirming at least one unstained or single-stain control, use the Set Up Matrix icon in the Sample Manager panel to perform spectral unmixing of the recorded fluorescence data, which generates the spectral unmixing matrix. The spectrally unmixed data can be viewed on the View Data page and Sort page (BD FACSDiscover™ S8 Cell Sorter only).

**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.

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

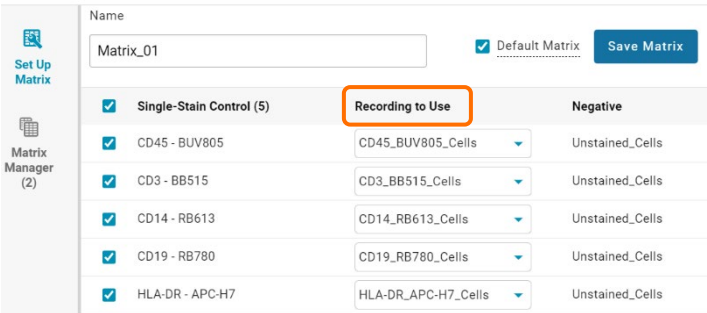
Performing spectral unmixing

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

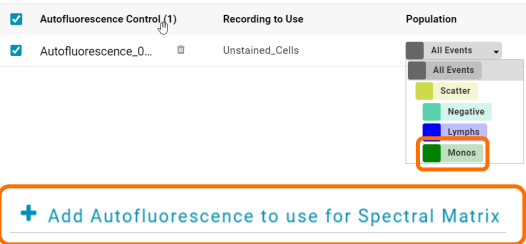


2. Select the recording to use for each fluorescent single-stain control.

Set Up Spectral Matrix

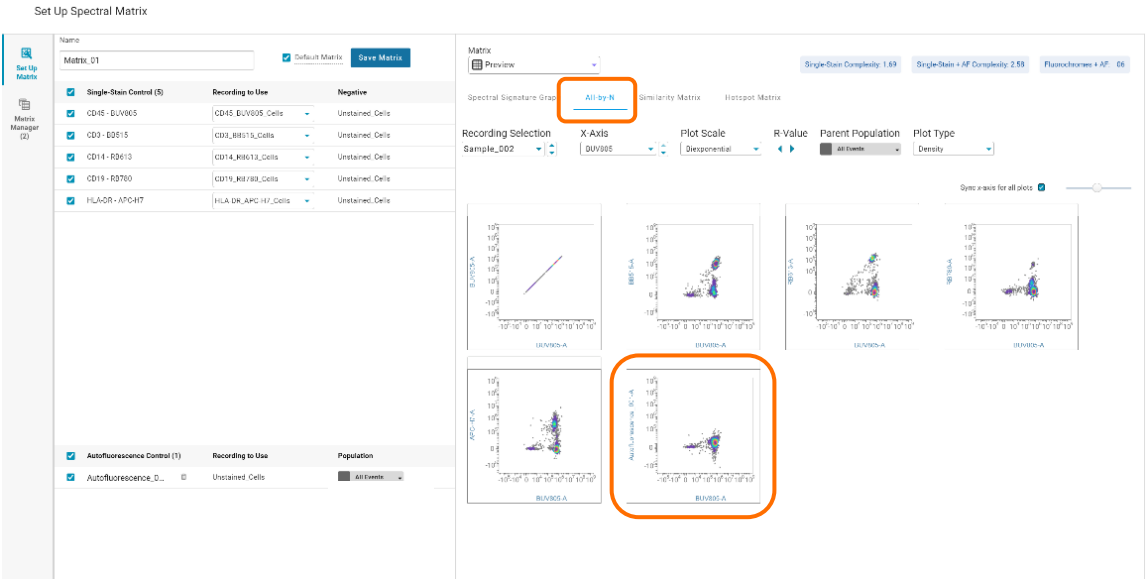


3. (Optional) Click **Add Autofluorescence Control** in the bottom left and select the unstained control to use for unmixing autofluorescence.



4. Click **All-by-N** to preview a visual overview of the data.  
The Matrix Preview displays the single-stained controls as All-by-N plots.

An autofluorescence control plot is displayed for each included autofluorescence control.



Performing spectral unmixing, continued

5. Enter a name and click **Save Matrix**.

**NOTE** By default, the system assigns a name and the number increases depending on the number of matrices in the system.

Set Up Spectral Matrix

Set Up Matrix

Matrix Manager (0)

Name

Matrix\_01

☒ Default Matrix

Save Matrix

Single-Stain Control (3)	Recording to Use	Negative
<input checked="" type="checkbox"/> viability - FVS450	viability_FVS450_001	In-Tube
<input checked="" type="checkbox"/> GFP - eGFP	eGFP_001	In-Tube
<input checked="" type="checkbox"/> DNA - DRAQ5*	DNA_DRAQ5_001	In-Tube

6. Confirm that a matrix was generated with a timestamp.

+ Add Autofluorescence to use for Spectral Matrix

✔ 'Matrix\_01' successfully saved to Matrix Manager as a Default Matrix 09/17/2025 04:46:11 PM

7. (Optional) Click the **Matrix Manager** icon to view the saved matrix in the Matrix Catalog.

Set Up Spectral Matrix

Set Up Matrix

Matrix Manager (2)

Matrix Catalog (2)



Default Matrix

Matrix\_01

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8. Click **Close** in the lower right of the window to exit the Set Up Spectral Matrix window.

Unmixing tips

Component	Page	Function
Importing controls <div></div>	Set Up Single-Stain Controls	Allows you to import controls from one or more experiments into your current experiment. <ul style="list-style-type: none"><li>You can only import controls from one or more experiments that were created by your user account.</li><li>You cannot import controls created on another instrument with a different optical configuration.</li></ul>
Assign Matrix <div></div>	View Data	Allows you to assign different spectral matrices to different samples or controls in an experiment. Spectral matrices that were set up on an instrument with a different optical configuration cannot be applied to new samples on the current instrument, but can still be applied to samples that were recorded on the same instrument.

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