

## **BD FACSuite<sup>™</sup> Software**

Quick Start Guide for Common Workflows

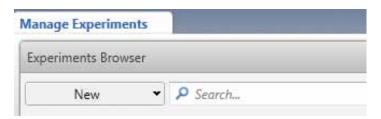
- Create an Experiment
- Create an Assay
- Worklist Workflow
- **Setup Workflows** (on reverse)

## **Create an Experiment**

Before running samples, an experiment should be created in the Experiment workspace. There are three ways to create an experiment.

#### 1) Create a new experiment

- a. Select the **Experiments** workspace from the navigation pane.
- b. Select **New** in the Experiment Browser.



A new experiment tab will open in the Experiment workspace with a default tube. By default, this tube is created using Lyse Wash Settings.

#### 2) Create an experiment from an assay

- a. Select the **Experiments** workspace from the navigation pane.
- b. Select **New from Assay** within the Experiment Browser.
- c. Select an assay from the drop-down menu.

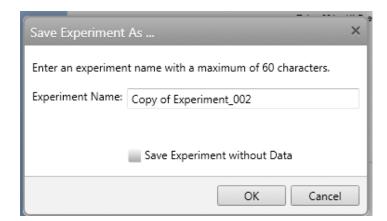


A new experiment is created with the name of the selected assay.

## **Create an Experiment (continued)**

#### 3) Duplicate a saved experiment

- a. Select the **Experiments** workspace from the navigation pane.
- b. Select and right-click an existing experiment.
- c. Select **Save As**.
- d. Name the experiment. If applicable, select the **Save Experiment without Data** checkbox.



Note: Once you have created the experiment, refer to the Setup Workflows section and select an appropriate workflow for your experiment.

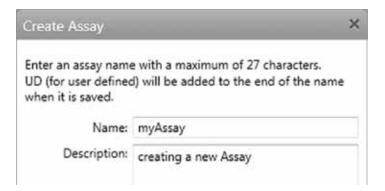
Once you create an experiment, you can create an assay from it.

## **Create an Assay**

Creating an assay is required to run the samples using a worklist. Once you create an experiment, you can create an assay from it.

#### 1) Create an Assay

- a. Open the experiment you plan to use to create an assay.
- b. Go to File > Create Assay.
- c. Name the assay.

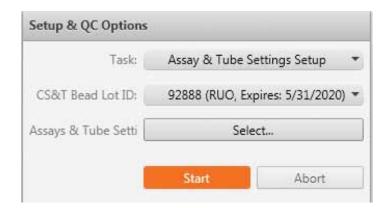


Note: To create an assay, all tubes have to be associated with saved Tube Settings.

## **Create an Assay (continued)**

#### 2) Update Assay and Tube Settings Setup

- a. Go to Setup & QC.
- b. Select **Assay & Tube Setting Setup** from the Task list.
- c. Select CS&T Bead Lot ID.
- d. Click **Select**.
- e. Select the assay you want to update.
- f. Click **Start**.



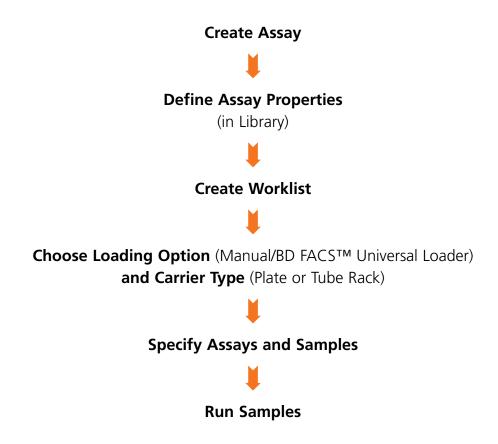
Note: This function updates Assay and Tube Settings based on current Performance QC. This update ensures that if the performance of your cytometer changes over time, your results will be consistent from experiment to experiment. To access this function, go to Setup & QC.

#### 3) Define Assay Properties

- a. On the navigation pane, select the **Library** workspace and go to **Assay**.
- b. Find your assay under User-Defined.
- c. Select your assay and go to the **General**, **Export Results**, and **Report** tabs to make necessary changes.

The next step is to create a Worklist.

#### **Worklist Workflow**

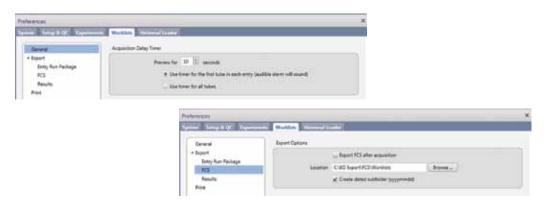


#### **Worklist Workflow**

This workflow can be used for both a tube rack and a plate.

#### 1) Set worklist preferences

- a. On the menu bar, go to **Tools** > **Preferences** > **Worklists**.
- b. Specify General, Export, and Print preferences for the worklist workspace.



#### 2) Create a new worklist

- a. Go to the **Worklist** workspace.
- b. On the menu bar, go to **File** > **New Worklist**.

#### 3) Select a Carrier Type

a. In the **Loading Options** panel, select the carrier type.



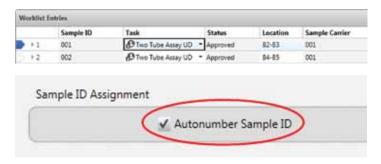
Note: If needed, tubes may be loaded manually.



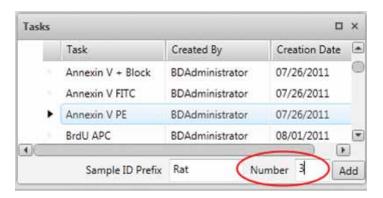
b. Create worklist entries by entering Tasks (Assays) and Sample IDs.

#### 4) Create worklist entries

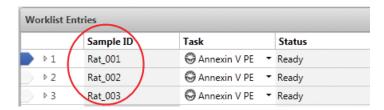
To specify a prefix for sample IDs in worklist entries, select the **Autonumber Sample ID** option in worklist preferences and follow these steps.



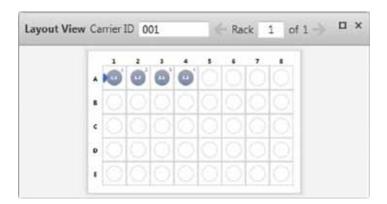
a. In the **Tasks** panel, select a task to add to the worklist.



- b. In the **Sample ID Prefix** field at the bottom of the panel, enter a name.
- c. In the **Number** field, enter the number of tasks you want to add and click **Add**.
- d. The tasks are added to the worklist with the prefix you entered.

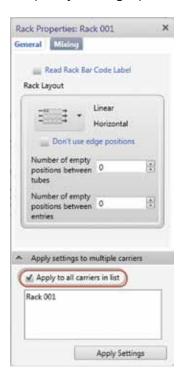


- 5) Specify Layout and mixing options in Properties
- a. In the Layout View, right-click and select Display Properties.



b. Specify Layout options on the **General** tab. When the carrier is a plate, the notch positions on the plate must match the notch positions in the **Plate Properties**. Choose the location of the notch placement.

c. Specify mixing options on the Mixing tab.





Note: Select **Apply to All** to apply layout and mixing options to multiple carriers.

#### 6) Run samples

The following options are available to run samples:

- a. Run All will run all samples on the carrier.
- b. **Run from Pointer** will run all samples starting from the selected sample.
- c. **Run Selected** will run the samples that are selected in the Layout View.

To increase the preview time between samples, use the Stop Timer button as needed.

Note: By default, the preview time is set to 10 seconds. To modify the default value, go to **Tools** > **Preferences** > **Worklist** tab on the menu bar.

#### 7) Batch analysis

After data acquisition, using batch analysis in the Worklist Workspace, you can reanalyze data, reprint reports, and export statistics.

The Run buttons are the controls for batch analysis.

The Stop Timer button can be used to pause between data files.

## Setup Workflows =

For step-by-step instructions on how to choose and complete a Setup Workflow, please turn this Quick Start Guide around and follow the instructions.

## **Setup Workflows: Overview**

From the following five options, choose the setup workflow that matches your needs.

| Workflow                                      | Scenario  | What is required?                   |  |
|---|---|-------------------------------------|--|
| <b>Default</b> (Lyse Wash)                    | Use when no adjustments need to be made to PMTVs and compensation values.   | No action required                  |  |
| Custom 1<br>(Modify Lyse Wash)                | Use when adjustments to PMTVs, including FSC or SSC, are necessary, but the experiment will not be repeated.                            | Adjust PMTVs as desired             |  |
| Custom 2<br>(Modify Lyse Wash and Save)       | Use when adjustments to PMTVs, inclusing FSC or SSC, are necessary, and the experiment will be repeated.                                | Create Tube Settings                |  |
| Custom 3<br>(User-Defined Reference Settings) | Use when customized PMTVs and compensation values are necessary or if fluorochromes that do not exist in the spillover matrix are used. |                                     |  |
| Custom 4 (Save Modified Reference Settings)   | Use when existing Reference<br>Settings require adjustments, and<br>the experiment will be repeated.                                    | Save modified Reference<br>Settings |  |

## **Setup Workflows: Step-by-step**

Each setup workflow has a unique set of steps.

| Default<br>(Lyse Wash) | Custom 1<br>(Modify Lyse<br>Wash) | Custom 2<br>(Modify Lyse<br>Wash and<br>Save) | Custom 3<br>(User-Defined<br>Reference<br>Settings)   | Custom 4<br>(Save<br>Modified<br>Reference<br>Settings) |
|------------------------|-----------------------------------|---|---|---|
| Create tube            | Create tube                       | Create tube                                   | Create tube   | Select tube   |
|                        | Optimize PMTVs                    | Optimize PMTVs                                | Optimize PMTVs  | Adjust compensation                                     |
|                        |                                   | Create Tube<br>Settings                       | Create user-defined<br>Reference Settings<br>by acquiring single-<br>color compensation<br>controls | Save Modified<br>Reference Settings                     |
| Acquire data           | Acquire data                      | Acquire data                                  | Acquire data  | Acquire data  |

## **Default (Lyse Wash) Workflow**

Use this workflow when no adjustments need to be made to default PMTVs and compensation values. The default tube setting called Lyse Wash is available in the BD FACSuite Library Lyse Wash (settings are designed for white blood cells but may work for many cell types).

#### 1) Create a tube in an experiment

Default Lyse Wash Tube Settings are automatically assigned to a new tube.

#### 2) Label reagents as needed

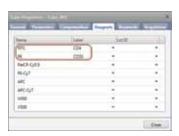
Specify reagent labels. Note: You can add, remove, or modify parameters (detectors) as needed.

#### 3) Set acquisition criteria

Set acquisition criteria including Select Time Stopping Rule or Gate Criteria, if needed.

#### 4) Acquire data







## **Custom 1: Modify Lyse Wash**

Use this workflow if modifications are needed to the PMTVs and you do not want to save the modifications. This workflow is ideal for experiments created for one-time use.

#### 1) Create a tube in an experiment

Default Lyse Wash Tube Settings are automatically assigned to a new tube.

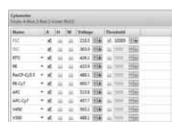
#### 2) Optimize PMTVs

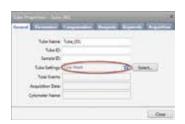
In this step, you can optimize PMTVs while previewing your cell sample in a worksheet. As PMTVs are adjusted, BD FACSuite software automatically adjusts spillover values.

You can add, remove, or modify parameters (detectors) as needed.

Note: The blue star appears when one or more of these elements are modified—PMTVs, Threshold, Flow Rate, Area Scaling Factor, or Window Extension.







## **Custom 1: Modify Lyse Wash (continued)**

## **3) Go to Tube Properties > Reagents tab** Label reagents as needed.

## **4) Go to Tube Properties > Acquisition tab** Specify Time Stopping Rule and Gate Criteria as needed.

#### 5) Acquire data

Note: To create additional tubes using the same settings, there are three different methods:

- 1. Use the **Next** button;
- 2. Use the **Duplicate without data** option; or
- 3. Use the **New Tube** button and choose the Tube Settings in the Tube Properties.

## **Custom 2: Modify Lyse Wash and Save**

Use this workflow when adjustments are needed to PMTVs and the experiment will be repeated in the future. This workflow is ideal for routine experiments that will be repeated using the same settings.

#### 1) Create a tube in an experiment

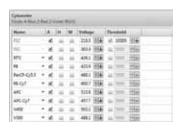
Default Lyse Wash Tube Settings are automatically assigned to a new tube.

#### 2) Optimize PMTVs

In this step, you can optimize PMTVs while previewing your cell sample in a worksheet. As PMTVs are adjusted, BD FACSuite software automatically adjusts spillover values.

Note: Select Preview to view your sample while modifying the settings. Be sure not to acquire data in the tube before creating your Tube Settings. Tube Settings cannot be created from a tube that contains saved data. If you do record data, there is a Clear Tube option to use before creating Tube Settings.







# Custom 2: Modify Lyse Wash and Save (continued)

- **3) Go to Tube Properties > Reagents tab** Label reagents as needed.
- **4) Go to Tube Properties > Acquisition tab**Specify Time Stopping Rule and Gate Criteria as needed.
- 5) Acquire data

Note: To create additional tubes using the same settings, there are three different methods:

- 1. Use the Next button;
- 2. Use the **Duplicate without data** option; or
- 3. Use the **New Tube** button and choose the Tube Settings in the Tube Properties.

On subsequent days, update your Tube Settings using the Assay & Tube Settings Setup option in the Setup & QC Workspace.

## **Custom 3: User-Defined Reference Settings**

Use this workflow when customized PMTVs and compensation values are necessary or if fluorochromes that do not exist in the existing spillover matrix are used.

#### 1) Create a tube in an experiment

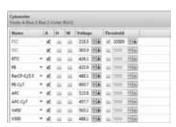
Default Lyse Wash Tube Settings are automatically assigned to a new tube.

#### 2) Optimize PMTVs

In this step you can optimize PMTVs while previewing your cell sample in a worksheet. As PMTVs are adjusted, BD FACSuite software automatically adjusts spillover values.

Note: Select Preview to view your sample while modifying the settings. Be sure not to acquire data into the tube before creating your Tube Settings. Tube Settings cannot be created from a tube that contains saved data. If you do record data, there is a Clear Tube option to use before creating Tube Settings.





# Custom 3: User-Defined Reference Settings (continued)

- 3) Add a lot-specific reagent to the Library (Optional)
- a. Go to Library > Beads & Reagents > Reagents.
- b. Select **Add** to add a reagent.
- c. Specify the following for the reagent:
  - Product Type
  - Single Color (checkbox)
  - Product Name (marker + fluorochrome)
  - Fluorochrome
  - Label (marker)

Note: To create additional tubes using the same settings, there are three different methods:

- 1. Use the **Next** button;
- 2. Use the Duplicate without data option; or
- 3. Use the **New Tube** button and choose the Tube Settings in the Tube Properties.





# Custom 3: User-Defined Reference Settings (continued)

#### 4) Create Reference Settings

- a. Right-click on a tube within the experiment and select **Create Reference Settings**.
- b. Add the fluorochrome of interest.
- c. Specify the Label and Lot ID as needed.
- d. Select the location of Unstained Reference Particles.



Note: Select FC (Fluorescence Control) if using cells.

- e. Click the **Next** button to acquire the fluorescence controls.
- f. After acquisition, name the Reference Settings and click **Finish** to create Reference Settings.

#### 5) Go to Tube Properties > Reagents tab

Label reagents as needed.

#### 6) Go to Tube Properties > Acquisition tab

Specify Time Stopping Rule and Gate Criteria as needed.

#### 7) Acquire data

Note: To create additional tubes using the same settings, there are three different methods:

- 1. Use the **Next** button;
- 2. Use the **Duplicate without data** option; or
- 3. Use the New Tube button and choose the Tube Settings in the Tube Properties.

On subsequent days, update your Tube Settings using the Assay & Tube Settings Setup option in the Setup & QC Workspace.

## **Custom 4: Save Modified Reference Settings**

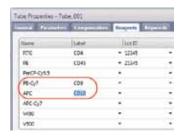
Use this workflow when existing reference settings require adjustments and the experiment will be repeated.

1) Select a tube within the experiment Right-click on the tube and select **Tube Properties**.

#### 2) Remove labels

Remove non-lot–specific labels assigned to parameters for which compensation will be adjusted.

- **3) Adjust the compensation** Adjust compensation values as needed.
- **4) Select Save Modified Reference Settings**Right-click on the tube within the experiment and select **Tube Properties**. Then select **Save Modified Reference Settings**.





# Custom 4: Save Modified Reference Settings (continued)

#### 5) Name Reference Settings

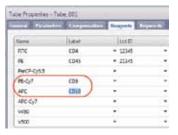
In the **Name** field, provide a name for the Reference Settings to be modified.

Note: If you began with Lyse Wash/Lyse No Wash (LW/LNW) Reference Settings, you must give them a new name. It is helpful to supply a meaningful description to help differentiate the new Reference Settings from others.

#### 6) Select Finish

Note: Modified Reference Settings are saved in the Library with associated Tube Settings and adjusted compensation.

- 7) Add any labels back to the tube properties
- 8) Acquire data



On subsequent days, update your Tube Settings using the Assay & Tube Settings Setup option in the Setup & QC Workspace.

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