BD FACS Sample Prep Assistant III

- Tutorial 1: Using BD Multitest Panels with the SPA III on page 3
- Tutorial 2: Using Custom Panels with the SPA III on page 15
Tutorial 1

Using BD Multitest Panels with the SPA III

After completing this tutorial, you will be able to:

- Create and run a worklist for a BD Multitest panel.
- Use different primary tube types on the same primary tube rack.
- Prepare a second worklist while running the first.
- Incubate samples offline.
- Import a worklist into BD FACSCanto clinical software.
Before You Begin

Tutorial Requirements

Use BD FACSTM SPA software and BD FACSCanto™ clinical software to perform this tutorial.

Ensure that a user account has been created for you by your system administrator.

NOTE This tutorial is performed without an instrument. If an instrument is connected to your workstation, ensure that it is turned off before you start SPA software.

1 Turn on the computer and double-click the BD FACS SPA Software shortcut on your desktop.

   The welcome screen is displayed, followed by the login dialog.

2 Select your user name from the menu and type your password.

3 Click OK.

   The Prep Worklist window opens.
Setting Up a Worklist

In this section, you will create a worklist and specify sample IDs, primary tube types, sample tube positions, panels, and carousel rack positions for the secondary tubes. You will also select a reagent rack appropriate for staining all the tubes in the selected panels.

SPA software uses this information to direct the instrument during sample preparation. Worklists can be saved, re-opened, and printed.

Entering Sample Information

1. Click in the text field directly below the Sample ID heading and type Control.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Sample ID</th>
<th>Case Number</th>
<th>Primary Tube Type</th>
<th>Primary Rack Position</th>
<th>Panel Name</th>
<th>Carousel Position</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample 1</td>
<td></td>
<td>Primary Rack 1</td>
<td>BD Vacutainer</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

The sample is automatically assigned to Primary Rack Position 1. The primary tube rack display highlights position 1, signifying the position of this primary tube.

The panel name defaults to the last setting from the previous worklist.

TIP A Sample ID is required for every sample entry in a worklist. Other sample information (eg, sample name and case number) is optional.

TIP Resize columns in the worklist by placing the cursor in the column title and dragging to the right or left.

2. Press the Tab key twice to advance to the Primary Tube Type field.

3. Use the Primary Tube Type menu to specify BD Vacutainer as the primary tube type.

Selecting the incorrect tube type can cause incorrect results and/or damage to the instrument during operation.

4. Press the Tab key twice to advance to the Panel Name field.
5 Use the Panel Name menu to select 6 Color TBNK as the panel name.

6 Color TBNK is a one-tube panel. Therefore, the secondary tube for this panel is assigned to Carousel Position 1.

The Secondary Tubes Carousel Position and Name field shows the tube in the panel.

Carousel rack position 1 is highlighted, signifying the location of the secondary tube.

6 Complete the remainder of the worklist, so that it looks like the following figure.
Making Worktable Assignments

In this section, you will select a reagent rack that includes all of the reagents required for the 6 Color TBNK panel. In addition, you will create a unique carousel ID and select offline incubation.

1. Select BD 6 Color from the Reagent Rack ID menu.

R1 on the Reagent Rack Map on the worktable display is highlighted, indicating that reagent is required in that position.

To view the name of the reagent associated with a reagent rack position, place the cursor over the highlighted position in the Reagent Rack Map. The reagent name is displayed.

2. Select 1 from the Carousel ID menu to specify a BD FACS™ Loader carousel rack ID.

   **NOTE** The carousel rack ID number is located on top of the BD FACS Loader carousel rack.

3. Type Training into the Unique Carousel ID field.

   **NOTE** The unique carousel ID can also be entered using the barcode reader.

4. Select the Incubate Offline checkbox.

This feature allows you to self-monitor the incubation of your samples with lyse solution. By removing the initial carousel rack, you are able to begin processing another worklist, thereby increasing your throughput.
Entering Lot Information

With SPA software, you can enter lot information for reagents, BD Trucount™ beads, and control beads. In this section, you enter simulated lot information for the BD 6 Color reagent.

1. Select Setup > Lot IDs.

The Lot IDs dialog opens.


3. Click OK to accept the lot information and close the dialog.

Lot IDs are saved with a worklist.
Running a Worklist

1. Click Run.

   A verification dialog opens. The dialog requests that key requirements are verified. It also specifies the volume of the reagents needed for the run.

   ![Verification Dialog]

2. Complete the verifications, then click OK.

3. Click OK again to save the worklist.

   ![Save Worklist]

   Choose a location to save the worklist and name it Multitest Training.

   A saved worklist can be opened from the File menu in the Prep Worklist window and re-used.
Sample Processing

Sample processing begins with some instrument checks, followed by reagent dispensing. As samples are processed:

- The Status text area describes the steps of the process.
- Rack positions in the worktable display are highlighted to indicate the tubes and vials involved in each step.
- The status bar at the bottom of the Prep Worklist window displays the cumulative number of probe pierces.

Preparing a Second Worklist

While you are running one worklist, you can prepare another.

1 Select File > New Prep Worklist.

A new, second worklist opens in the Prep Worklist window. You can now enter sample information on the second worklist as the first worklist runs.

2 (Optional) Enter a sample on the second worklist by duplicating the steps in Entering Sample Information on page 5.

NOTE Whenever user action is required for the worklist being run, a dialog appears.
3 To monitor the status of the run, display the running worklist by clicking its tab.

4 Return to the second worklist by clicking its tab.

In this tutorial, you do not run a second worklist. At this time, close the second worklist without saving it, and return to the running worklist.

5 Click Close.

If you have entered information in the second worklist, a dialog opens asking if you want to save it.

6 Click No.
Completing the Run

1  Click OK to complete the SPA run.

2  The End Prep Worklist Execution dialog opens. Choose whether to print.
   - If a printer is installed, click Print.
   - Otherwise, click Close.

Shutting Down the Software

1  Click Shutdown.

   The shutdown dialog opens.

2  Click OK.

   The power off dialog opens.

3  Click OK.
Transferring the Worklist to a BD FACSCanto II Workstation

**NOTE** If you are transferring the worklist to BD™ Worklist Manager or BD FACSDiva software, see the *BD FACS Loader User’s Guide* or the *BD FACSDiva User’s Guide* for more information.

**IMPORTANT** To import the worklist, all reagent and panel names must exactly match those used in BD FACSCanto clinical software. Sample information must be imported into a new, empty worklist. You cannot add imported information to a worklist that is already started.

2. Plug the USB device into the BD FACSCanto II workstation.
3. Start BD FACSCanto clinical software.
4. Select *File > Import SPA Worklist*.
5. Navigate to your USB device and select *Multitest Training*.
6. Click *Open*.
    The SPA worklist is imported into the BD FACSCanto clinical software.
7. Click .

**NOTE** In order to complete steps 7 and 8 in the tutorial, you must turn on your cytometer and complete fluidics startup.
8 After being prompted to save the worklist, you will encounter the following dialog.

![SPA Unique Carousel ID](image)

- Enter *Training* in the **Unique Carousel ID** field.
- Click **Cancel**. If you were continuing with the run, then you would click **Continue**.

9 Select **File > Exit** to close the software.
Tutorial 2

Using Custom Panels with the SPA III

After completing this tutorial, you will be able to:

- Create a custom panel.
- Create a custom reagent rack.
- Create and run a worklist using a custom panel.
- Import a panel into BD FACSDiva software.

**NOTE**  All reagent volumes, lyse volumes, and incubation times in this tutorial are for training purposes only.
Before You Begin

Tutorial Requirements

Use SPA software and BD FACSDiva software to perform this tutorial.

Ensure that you have access to a user account with administrative privileges (only necessary for SPA software).

**NOTE** This tutorial is performed without an instrument. If an instrument is connected to your workstation, ensure that it is turned off before you start SPA software.

1. If necessary, start SPA software.
2. Select your user name from the menu and type your password.
3. Click OK.

The Prep Worklist window opens.

**NOTE** If you are continuing from a previous software session, ensure that your worklist is clear.

To start a new worklist, select File > New Prep Worklist.
Creating a Custom Panel Using SPA Software

SPA software allows you to create your own customized panels and modify preprogrammed variables for each tube in your panel. You can also customize reagent and lyse volumes, as well as reagent and lyse incubation times. In addition, custom reagents can be added to the Reagent List.

In this tutorial, you will create a three-tube custom panel that utilizes multiple reagent racks.

Panel Setup

Creating a Category

1. From the main menu in the Prep Worklist window, select Setup > Panel Editor.

   The Panel Editor is displayed.

2. Click New Category.

   A New Category folder is listed below the existing panel category folders in the browser pane on the left.
3 Enter a name for the new panel category.
   a Right-click the new category and select Rename.
   b Change the name of the new category to Tutorial Panels.

   **NOTE** User-defined category, panel, and tube names are displayed in blue. Pre-programmed (non-editable) categories, panels, and tubes are black.

4 Verify that the Tutorial Panels folder is still selected and click New Panel.

   A new panel, Untitled-Panel, is listed in the browser pane.

5 Change the name of the new panel to Practice.

6 Select the Other Panel button.
Creating Secondary Tubes for the New Panel

Creating and Modifying Tube 1

1 In the Panel Editor, click to select *Practice*.

2 Click New Tube.

A new tube, *Untitled tube*, is listed in the browser pane.

3 Change the name of the new tube to *Tube 1*.

4 Verify that *Tube 1* is still selected and click Edit Tube.

The Tube Editor is displayed.
5 Click the up or down arrows to change Tube Editor settings to the following:

- Sample Volume (µL): 100
- Lyse Volume (µL): 0

6 Click the **Reagent List** link.

The Reagent List is displayed. The Reagent List contains all of the BD pre-programmed reagents and any custom reagents previously created. New reagents can be added to this list, and they will be available for later use. Any reagent name on this list can be dragged to the **Reagent** field in the Tube Editor.

7 Add a new reagent to the Reagent List.

   a Click in the **Enter a Custom Reagent Name** field.

   b Type Reagent 1.

   c Click Add.

   Reagent 1 is now added to the Reagent List.

8 Click and drag Reagent 1 from the Reagent List to the **Reagent** field in the Tube Editor.

9 Repeat steps 7 and 8 to add Reagents 2–9 to the **Reagent** field in the Tube Editor.

10 Close the Reagent List.
11 Select *Reagent 1* in the *Reagent* field of the Tube Editor and change the *Volume (µL)* to *15*.

12 Select each reagent and change its volume to *15*.

13 In the Tube Editor, click *Apply* to apply the current settings to Tube 1.

14 In the Tube Editor, click *Close*. 
Creating additional secondary tubes

You will create two additional secondary tubes for a total of three tubes in the Practice panel.

Creating Tube 2

1. Add a new tube to the Practice panel and name it Tube 2.

![Panel Editor]

2. Click Edit Tube to edit Tube 2 settings.

   **TIP** Double-clicking a tube also opens the Tube Editor.

3. Change Tube Editor settings to the following:
   - Sample Volume (µL): 100
   - Lyse Volume (µL): 0

4. Click the Reagent List link.

5. Add Reagents 10–18 to the Reagent List.

6. Drag the reagents to the Reagent field in the Tube Editor.

7. In the Reagent field in the Tube Editor, change the reagent volumes to 15.
8 Click **Apply** to apply the current settings to Tube 2.

9 In the Tube Editor, click **Close**.

Both the Tube Editor and the Reagent List close.
Creating Tube 3

In this section, you will create a new tube by making a copy of the previous tube and then editing its contents.

1. Create a copy of Tube 2:
   
   a. Right-click Tube 2 in the Panel Editor, then select Copy from the menu.
   
   b. Right-click again and select Paste from the menu.

   A new tube, Copy of Tube 2, is added to the Practice panel tubes.

2. Change the name of the new tube to Tube 3.

3. In the Panel Editor, click Edit Tube.

4. Verify the following Tube Editor settings:
   
   - Sample Volume (µL): 100
   - Lyse Volume (µL): 0

5. Select each of the reagents in the Tube Editor reagent field and press the Delete key to delete these reagents from the reagent field.

6. Click the Reagent List link.


8. Drag the reagents to the Reagent field in the Tube Editor.

9. Change the reagent volumes to 15.
10 Click **Apply** to apply the current settings to Tube 3.

![Tube Editor](image)

11 In the Tube Editor, click **Close**.

Both the Tube Editor and the Reagent List close.
Modifying Incubation Settings

1. In the Panel Editor, select the Practice panel.
2. Set the Reagent Incubate time to 5 minutes.
3. Set the Lyse Incubate time to 0 minutes.
4. Click OK to close the Panel Editor.
Creating a Custom Reagent Rack

In this section, you will create a custom reagent rack that includes the reagents needed to stain the tubes in the Practice panel.

1 Select Setup > Reagent Rack Editor.

The Reagent Rack Editor is displayed.

2 Click the Custom Reagent Racks folder to select it.

3 Click New Rack.

A new rack, New Rack, is added to the Custom Reagent Racks folder.

4 Change the name of the new rack to Training 27.
5 Verify that the *Training 27* reagent rack is selected and click *Edit Rack*.

The Rack Map Editor is displayed.

6 Click the *Reagent List* link.

7 Drag *Reagent 1* to the *Reagent* field in the Rack Map Editor.

8 In the Rack Map Editor, click *R1* to select it as the location for the *Reagent 1* reagent vial.

**TIP** Moving the cursor over a highlighted reagent vial position displays the reagent name associated with that position.
9 Repeat steps 7 and 8 for Reagents 2–18. Your completed Rack 1 will look like the following figure.

![Rack Map Editor](image)

10 Click Add Rack.

A new reagent rack is added.

11 Click Next.

Reagents can now be positioned in Rack 2.
12 Drag reagents 19–27 from the Reagent List to the Rack Map Editor and assign them to positions R1–R9 in the Reagent Rack.

13 In the Rack Map Editor, click OK.

Both the Rack Map Editor and the Reagent List close.

14 Click OK in the Reagent Rack Editor.

The Reagent Rack Editor closes.
Using a Custom Panel in a Worklist

In this section, you will create and run a worklist using the panel you just created.

1. Ensure that you have a blank worklist open.
   - If necessary, select File > New Prep Worklist to open a blank worklist.
2. Type Donor 1 in the Sample ID field.
3. Verify that BD Vacutainer is selected as the Primary Tube Type.
4. Select Practice from the Panel Name menu.
5. Select Training 27 from the Reagent Rack ID menu.
6. Select 1 from the Carousel ID menu.
7 Click **Run**.

A verification dialog opens. The dialog requests that key requirements are verified. It also specifies the volume of the reagents needed for the run.

8 Complete the verifications, then click **OK**.

9 Click **OK** again to save the worklist.

Choose a location to save the worklist and name it *Custom Panel Practice*. 
After the sample has been dispensed, you will be prompted to place the first reagent rack in the instrument.

Click **OK**.

After all of the reagents from Reagent Rack 1 have been dispensed, you will be prompted to place the second reagent rack in the instrument.

Click **OK**.

The run continues until completion.

When the worklist is complete, the **End Prep Worklist Execution** dialog opens. Choose whether to print.

- If a printer is installed, click **Print**.
- Otherwise, click **Close**.
Shutting Down the Software

1  Click Shutdown.

   The shutdown dialog opens.

2  Click OK.

   The power off dialog opens.

3  Click OK.
Importing a Custom Panel into BD FACSDiva Software

NOTE If you are transferring the worklist to BD Worklist Manager software, see the BD FACS Loader User’s Guide for more information.

Prior to importing a SPA worklist into BD FACSDiva software, you must create a panel template (specimen and tubes) identical to the panel you created in the SPA software. For information on how to create panel templates, see the BD FACSDiva Software User’s Guide.

1 Copy the saved worklist, Custom Panel Practice, to a portable USB device.

2 Plug the USB device into the BD FACSCanto II workstation.

3 Start BD FACSDiva software.

4 Select File > Import > Worklist.

5 Navigate to the saved worklist, then click Import.

In the browser, a new experiment will be created containing your SPA worklist.

When a SPA worklist is imported into BD FACSDiva software, the worklist name becomes the experiment name and the sample ID becomes the specimen name.

NOTE If you have not created the Practice panel template in BD FACSDiva software, you will receive an error message.

6 Select File > Quit to close the software.