

BD Cytometric Bead Array
(CBA) Kit for the
BD FACSAarray Bioanalyzer
Instruction Manual



About this guide

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History

Revision	Date	Change Made
644860	10/08	Updated information and format
03-8100055-11	2003	New document

BD flow cytometers are class I (1) laser products

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Introduction

This section includes these topics:

- About using the BD FACSArray for BD CBA assays (page 6)
- Protocol workflow (page 8)

About using the BD FACSAArray for BD CBA assays

Overview

The BD FACSAArray™ bioanalyzer allows you to analyze samples directly from a 96-well plate. It is a fast, reliable, and easy-to-use system for analysis of multiplex immunoassays such as BD™ Cytometric Bead Array (CBA) kits.

Following acquisition on the BD FACSAArray bioanalyzer, data files are exported and analyzed using FCAP Array™ software, which comes installed on the BD FACSAArray workstation.

About this protocol

The protocol described in this guide is intended for:

- Performing BD CBA experiments in 96-well filter plates.
 - Setting up the BD FACSAArray bioanalyzer.
 - Analyzing data with FCAP Array software.
-

Materials required

The following materials are required for this protocol. However, not all of the materials are provided with this kit.

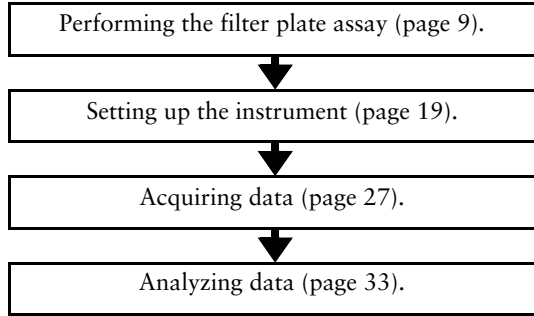
Material	Location
BD CBA kit	Purchase separately.
BD CBA application guide	Included in your BD CBA kit.
BD CBA single-color setup template	Download from bdbiosciences.com/cbatemplates .
Filter plates (For example, Millipore MultiScreenHTS-BV 1.2 m clear non-sterile filter plates)	Purchase separately from Millipore (millipore.com).

Material	Location
Vacuum Manifold (For example, Millipore MultiScreenHTS Vacuum Manifold)	Purchase separately from Millipore (millipore.com).
Shaker (For example, MTS 2/4 Digital Stirrer, IKA Works, VWR)	Purchase separately from IKA (ika.net).
Standard BD Falcon™ microtiter plate for BD FACSAarray bioanalyzer setup	Purchase from bdbiosciences.com (Cat. No. 353910).
Vacuum source	Provided by your facility.
Vacuum gauge and regulator (if not using the recommended manifold)	Provided by your facility.
FCAP Array software, version 1.0.1	This software comes with the BD FACSAarray bioanalyzer.

More information

See the *BD FACSAarray User's Guide* for more information.

**Protocol
workflow**



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Performing the filter plate assay

This section includes these topics:

- About the filter plate assay (page 10)
- Selecting a one-step or two-step incubation assay (page 11)
- Performing the one-step incubation assay (page 12)
- Performing the two-step incubation assay (page 15)

About the filter plate assay

About the assay Performing a BD CBA experiment in a 96-well filter plate is very similar to the protocol described in any BD CBA kit.

You can perform the protocol for kits that require one-step incubation or two-step incubation. See *Selecting a one-step or two-step incubation assay* (page 11) for more information.

Typical time required You should plan for approximately 6 hours (including incubation times) to complete this assay.

Selecting a one-step or two-step incubation assay

About this topic Before you begin the filter plate assay, see the following tables to determine if your kit requires a one-step or two-step incubation.

One-step incubation assays

Mat. No.	Description
552990	Human Chemokine Kit
551811	Human Inflammatory Cytokines Kit (supernatant protocol)
550749	Human Th1/Th2 Kit I
551809	Human Th1/Th2 Kit II
557800	NHP Th1/Th2 Kit
552364	Mouse Inflammation Kit
551287	Mouse Th1/Th2 Kit

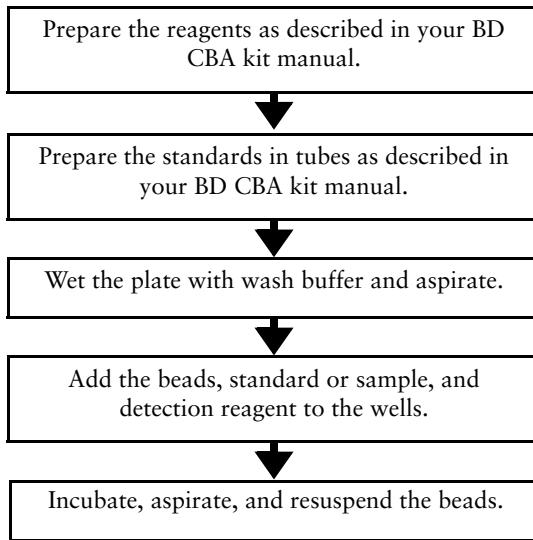
Two-step incubation assays

Mat. No.	Description
552363	Human Anaphylatoxin Kit
551811	Human Inflammatory Cytokines Kit (serum/plasma protocol)

Performing the one-step incubation assay

About this topic This topic describes the workflow and procedural steps for performing the filter plate assay with one-step incubation.

Procedure overview



Before you begin Set the vacuum pressure to 10" Hg or lower.

Procedure**To perform the assay:**

1. Wet the plate by adding 100 μL of wash buffer to each well.
2. Place the plate on the vacuum manifold.
3. Aspirate for 2–10 seconds until the wells are drained.
4. Remove the plate from the manifold, then blot the bottom of the plate on paper towels.
5. Add 50 μL of each of the following reagents to the filter plate:
 - Bead mix
 - Standard or sample
 - PE detection reagent
6. Cover the plate and shake it for 5 minutes at 1,100 rpm on a plate shaker.
7. Incubate the plate for the specified time.

Kit	Incubation time
<ul style="list-style-type: none"> • Human Inflammatory Cytokines kit for supernatant • Human Chemokine kit • Human Th1/Th2 kits • NHP Th1/Th2 kit 	3 hours
Mouse kits	2 hours

Note: The Mouse Ig Isotyping Kit cannot be analyzed on the BD FACSAarray bioanalyzer due to the use of a FITC detection reagent.



Caution! Place the plate on a non-absorbent, dry surface during incubations. Absorbent or wet surfaces can cause the contents of the wells to leak.

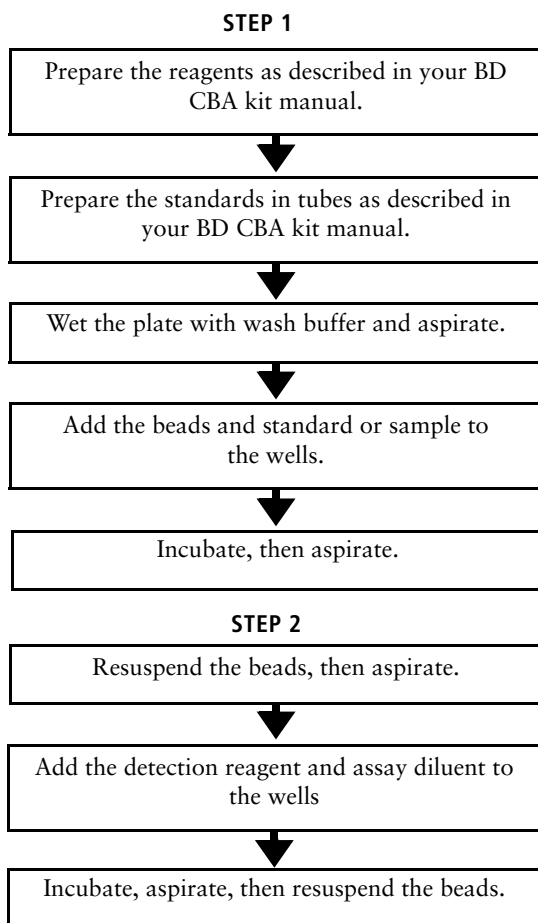
8. Remove the plate cover from the plate and apply the plate to the vacuum manifold.
9. Vacuum aspirate for 2–10 seconds until the wells are drained.
10. Remove the plate from the manifold, then blot the bottom of the plate on paper towels after aspiration.
11. Add 120 μL of wash buffer to each well to resuspend the beads.
12. Cover the plate and shake it for 2 minutes at 1,100 rpm before you begin sample acquisition.

Your assay procedure is complete.

Performing the two-step incubation assay

About this topic This topic describes the workflow and procedural steps for performing the filter plate assay with two-step incubation.

Procedure overview



Before you begin Set the vacuum pressure to 10" Hg or lower.

Procedure:
Step 1

To perform the first step of the two-step incubation assay:

1. Wet the plate by adding 100 µL of wash buffer to each well.
2. Place the plate on the vacuum manifold.
3. Aspirate for 2–10 seconds until the wells are drained.
4. Remove the plate from the manifold, then blot the bottom of the plate on paper towels.
5. Add 50 µL of each of the following reagents to the filter plate:
 - Bead mix
 - Standard or sample
6. Cover the plate and shake it for 5 minutes at 1,100 rpm on a plate shaker.
7. Incubate the plate for the specified time.

Kit	Incubation time
Human Inflammatory Cytokines kit for serum/plasma	1.5 hours
Human Anaphylatoxin kit	1 hour



Caution! Place the plate on a non-absorbent, dry surface during incubations. Absorbent or wet surfaces can cause the contents of the wells to leak.

8. Remove the plate cover from the plate and apply the plate to the vacuum manifold.

9. Vacuum aspirate for 2–10 seconds until the wells are drained.
10. Remove the plate from the manifold, then blot the bottom of the plate on paper towels after aspiration.
11. Add 200 μL of wash buffer to each well, cover the plate, and shake it for 2 minutes at 1,100 rpm.

Procedure:
Step 2

To perform the second step of the two-step detection assay:

1. Remove the plate cover from the plate and apply the plate to the vacuum manifold.
2. Vacuum aspirate for 2–10 seconds until the wells are drained.
3. Remove the plate from the manifold, then blot the bottom of the plate on paper towels after aspiration.
4. Add 100 μL of assay diluent to each well.
5. Add 50 μL of detection reagent to each well.
6. Cover the plate and shake it for 5 minutes at 1,100 rpm on a plate shaker.
7. Incubate the plate for the specified time.

Kit	Incubation time
Human Inflammatory Cytokines kit for serum/plasma	1.5 hours
Human Anaphylatoxin kit	2 hours



Caution! Place the plate on a non-absorbent, dry surface during incubations. Absorbent or wet surfaces can cause the contents of the wells to leak.

8. After the incubation, remove the plate cover from the plate and apply the plate to the vacuum manifold.
9. Vacuum aspirate for 2–10 seconds until the wells are drained.
10. Remove the plate from the manifold, then blot the bottom of the plate on paper towels.
11. Add 120 μL of wash buffer to each well to resuspend the beads.
12. Shake the plate for 2 minutes at 1,100 rpm before you begin sample acquisition.

Your assay procedure is complete.

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Setting up the instrument

This section includes these topics:

- Overview (page 20)
- Preparing the template (page 21)
- Preparing a plate (page 21)
- Creating a new experiment (page 22)
- Setting up the workspace (page 23)

Overview

When to set up the instrument

You can set up the BD FACSAArray bioanalyzer during the incubation of the assay.

Perform instrument setup for each type of BD CBA Kit you plan to run (Human Th1/Th2, Mouse Inflammation, etc.). Use the setup beads that are included with the BD CBA kit.

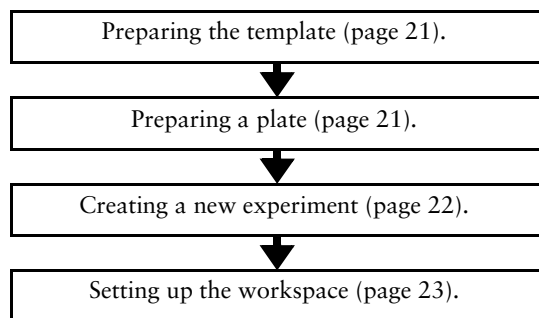
The instrument settings saved in each template are typically stable for up to a month. You must perform setup after the instrument is serviced.

Before you begin

Before you set up the instrument for the first time, download the *BD CBA Single Color Setup Template* from bdbiosciences.com/cbatemplates.

Workflow

Setting up the BD FACSAArray bioanalyzer includes these workflow stages.



Preparing the template

About this topic This topic describes how to prepare the template for instrument setup. You only need to download the template and install it once.

Procedure

To prepare the template for instrument setup:

1. On the BD FACSAria workstation, unzip the template and copy it into the templates folder.
2. In Windows® Explorer, navigate to C:\Program Files\BD FACSAria System Software\Templates.
3. Place a copy of the unzipped folder containing the BD CBA single-color setup template into the Templates folder.

Note: Do not edit the file name of the folder or the template.

Preparing a plate

About this topic This topic describes how to prepare a plate for instrument setup.

Procedure

To prepare a plate for instrument setup:

1. Add 50 μ L of Cytometer Setup Beads and 150 μ L of wash buffer to well A1 of a U-bottom plate.
 2. Mix by pipetting up and down several times.
-

Creating a new experiment

About this topic This topic describes how to create an experiment for instrument setup.

Procedure

To create an experiment for instrument setup:

1. In the BD FACSAArray system software, select **Experiment > Experiment Wizard**.

The **Experiment Wizard Welcome** dialog appears and quickly advances to the next view automatically.

2. Click **Default** for the **Wizard Session**, then click **Next**.
3. In the **Template** view, select **BD CBA Single Color Setup Template** from the menu, then click **Next**.
4. Click **Next** to apply the default values for the next 10 wizard views. These wizard views include:
 - Instrument setup and optical spillover
 - Number of calibrator standard replicates
 - Number of concentration levels
 - Concentration levels
 - Number of samples
 - Names of the samples
 - Number of sample replicates
 - Plate layout
 - Loader settings
 - Acquisition settings

You will return to some of these views in **Acquiring data** (page 27).

5. In the **Experiment Name** view, name the experiment. For example, Hu Th1Th2 Date, where Date is the current date (with no special characters). Click **Next** to go to the next screen.
6. In the **Saving Wizard Session** view, Click **No**, then click **Next**.
7. Review your selections in the **Completing the Experiment Creation Wizard**, then click **Finish**.

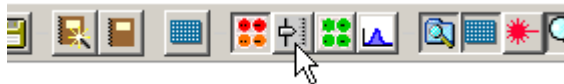
The **Prepare Workspace** view appears.

Setting up the workspace

About this topic This topic describes how to set up the workspace.

Procedure To set up the workspace in BD FACSAArray software:

1. Click the **Setup** button on the toolbar to display the **Setup Workspace** view.

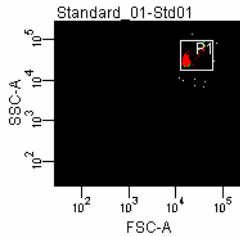


2. Place the plate containing the Cytometer Setup Beads on the plate holder, then click **Load in the Plate** window.
3. In the **Plate** window, click the **Acquire** tab to view the **Plate** layout.
4. In the **Plate** window, click **None**, then select well **A1**.
5. Click **Setup** under **Acquisition**.
6. Navigate to the **Instrument** window and click the **Parameters** tab.

7. In the **Instrument** window, adjust the FSC and SSC voltages so the beads are within the P1 gate and the bead aggregates are excluded from P1.

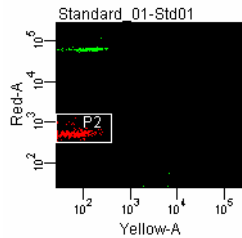
Alternatively, P1 can be moved over the beads provided that the bead singlets are at $>10^4$ in both FSC and SSC.

8. In the **Instrument** window, adjust the Red voltage so that the dim bead population falls within the P2 gate. With the dim beads in P2, the bright population should be approximately 10^5 .



If...	Then...
The dim beads do not fall within the P2 gate	Adjust the voltages in the Instrument window to move your population to the gate.
The P2 gate does not fit your population	Do not move or enlarge the P2 gate to fit your population. Adjust the voltages in the Instrument window to move your population to the gate.

- Adjust the Yellow voltage so that the dim bead population falls within P2 and the mean of the Yellow parameter is 30–50.



You might need to acquire the sample more than once in order to make all adjustments in the allotted time.

- Once acquisition stops, select **Experiment > Save as template** and save the template with a new filename.
-

4

Acquiring data

This section includes these topics:

- Overview (page 28)
- Starting data acquisition (page 30)
- Viewing BD CBA results (page 31)
- Exporting FCS 2.0 data files from an experiment (page 32)

Overview

About acquiring samples After you set up the instrument and save your template, you can begin acquiring samples. The following procedure uses the template you saved during instrument setup.

Before you begin You can verify instrument settings by preparing a well of setup beads and performing step 1 and step 3 in the following procedure to verify that the setup beads fall within the gates.

Procedure

To prepare for acquiring samples:

1. Click the **Experiment Wizard** button on the toolbar to open the Experiment Wizard.



2. In the **Template** view, select the template that you saved during setup, then click **Next**.

3. Enter the experiment-specific information in the following wizard views.

Wizard view	Setting
Enter the number of Concentration Levels	10
Enter the number of Samples	Set a value for your specific experiment requirements (for example, 25).
Loader Settings	<ul style="list-style-type: none"> ● Sample Flow Rate = 2.0 ● Sample Volume = 25 ● Mixing Volume = 60 ● Mixing Speed = 200 ● Number of Mixes = 1 ● Wash Volume = 200

4. Name the experiment. Click **Next** to go to the next screen.
 5. Select **No** in the **Saving Wizard Session** view and click **Next**.
 6. Review your selections in the **Completing the Experiment Creation Wizard** and click **Finish**.
The experiment opens.
-

Starting data acquisition

About this topic This topic describes how to use the BD FACSAArray bioanalyzer to acquire data.

Procedure To start data acquisition:

1. Place the assay plate into the plate holder and click **Load** in the **Plate** window.
2. Click **Acquire** under **Acquisition**.



Caution Region P2 is not used during acquisition. To prevent corrupting the template, do not delete P2.

If...	Then...
You see a large number of doublets	Adjust the P1 gate to include only the bead singlets.
You see debris	<p>Increase the FSC and SSC threshold values.</p> <p>Be careful not to set the thresholds so high that bead events are excluded. Use the “AND” Boolean operator to exclude events below the FSC threshold or the SSC threshold.</p>

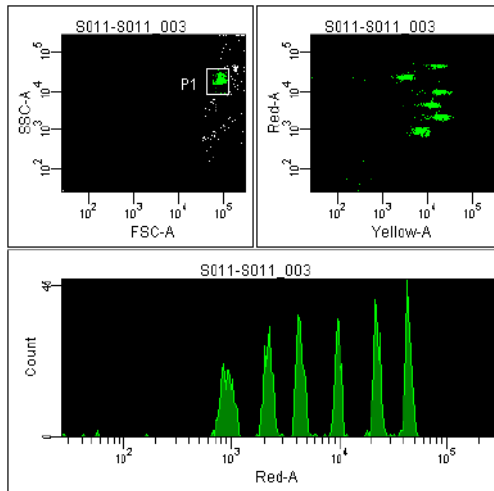
3. When acquisition completes, click **Unload** in the **Plate** window.
4. Click the **Prepare** button on the toolbar to display the **Prepare** workspace.



Viewing BD CBA results

About this topic This topic describes how acceptable BD CBA results should appear in plots.

Acceptable BD CBA results Populations in the dot plots should be clearly defined and tightly clustered. The peaks on the histogram should be single, well-separated peaks.



Exporting FCS 2.0 data files from an experiment

About this topic You can export data from an experiment as FCS 2.0 files if you want to analyze the data with different analysis software.

Procedure

To export data as FCS 2.0 files:

1. In the Browser, select the experiment that was just acquired.
 2. Select **File > Export > FCS**.
 3. Select **FCS 2.0**.
 4. Click **OK**.
 5. Click **Save** to export files to the default location.
-

5

Analyzing data

This section includes these topics:

- Overview (page 34)
- Creating a new experiment in FCAP Array software (page 35)
- Creating and adding bead groups (page 36)
- Defining clustering parameters (page 38)
- Assigning the analytes to bead populations (page 39)
- Selecting an experiment type (page 39)
- Specifying standards and analyte concentrations (page 40)
- Specifying the plate layout (page 41)
- Naming the experiment (page 41)
- Associating samples with data files (page 42)
- Starting analysis (page 43)
- Printing, reviewing, and exporting analysis data (page 44)

Overview

About analyzing data After you acquire data, you can use FCAP Array software to analyze the data.

Workflow Analyzing data includes these workflow stages.

Stage	Description
1	Creating a new experiment in FCAP Array software (page 35)
2	Creating and adding bead groups (page 36)
3	Defining clustering parameters (page 38)
4	Assigning the analytes to bead populations (page 39)
5	Selecting an experiment type (page 39)
6	Specifying standards and analyte concentrations (page 40)
7	Specifying the plate layout (page 41)
8	Naming the experiment (page 41)
9	Associating samples with data files (page 42)
10	Starting analysis (page 43)
11	Printing, reviewing, and exporting analysis data (page 44)

Creating a new experiment in FCAP Array software

About this topic This topic describes how to create a new experiment in FCAP Array software and how to add information (number of samples and dilution factors) specific to your assay.

Procedure

To create a new experiment:

1. Start FCAP Array software.
 2. Select **File > New Experiment Wizard**.
The **New Experiment Wizard** appears.
 3. Click **Next**.
 4. Specify the number of samples in the assay, then click **Next**.
 5. If appropriate, enter the dilution factor for each sample, then click **Next**.
-

Creating and adding bead groups

About this topic This topic describes how to create bead groups and add them to the library.

You only need to edit the Bead Library the first time you analyze a kit.

Procedure To create new bead groups and add them to the bead library:

1. In the **Selecting Saved Plex** view, click **New Plex**, then click **Next**.
2. In the **Plex Components** view, click **Edit** in the Beads pane.

The **Bead Library** window appears.

If...	Then...
You have already analyzed this kit in previous runs	Go to step 12.
This is the first time you are analyzing this kit	Go to step 3.

3. Click **Edit Groups**.
The **Bead Groups** window appears.
4. Click **New Group**.
The **New Bead Group** window appears.
5. Enter the name of the kit into the **New Bead Group** window, then click **OK**.
6. In the **Bead Groups** window, click **OK** to return to the **Bead Library** window.

7. In the **Bead Library** window, enter the information for the beads in the kit and click **Add** to add them to the Bead Library.

You can specify bead locations as 1 (dimmiest beads) to 6 (brightest beads). For more information, see Figure 1 in your BD CBA Kit manual.

8. After you enter all of the beads into the Bead Library, click **Edit Groups**.

The **Bead Groups** window appears.

9. Select the bead group you created, then click **Modify Group**.

10. Select the beads you want to add to the kit Bead Group by selecting the checkboxes. Click **OK** to add the beads.

The beads are now added to the designated bead group.

11. Click **OK** to close the **Bead Group** window, then click **OK** to close the **Bead Library** window.

12. In the left pane of the **Plex Components** view, double-click the name of the bead group to move its contents to the **Selected Beads** pane on the right.

13. Click **Next** to display the next view.
-

Defining clustering parameters

About this topic This topic describes how to define clustering parameters.

Procedure

To define the clustering parameters:

1. In the **Clustering Parameters** view, select **Load Data File** to navigate to the folder containing the experimental data files.
2. Select one data file from the folder and click **Select**.
3. Enter the following information from the lists:
 - Instrument name: BD FACSAArray
 - Scatter parameter: SSC-W
 - Number of scatter peaks: 1
 - Clustering parameters: Red-A and None
 - Reporter parameter: Yellow-A

The beads appear as a histogram in the data plot. If clustering was successful, the software displays a message in the bottom-left corner of the window.

4. Click **Next** to display the next view.
-

Assigning the analytes to bead populations

About this topic This topic describes how to assign analytes to bead populations.

Procedure To assign analytes to bead populations:

1. In the **Analyte Assignment** view, select an analyte in the table.
2. Double-click the corresponding bead peak to assign it.
Bead location 1 is the dimmest bead population.
3. Click **Next** to display the next view.

Selecting an experiment type

About this topic This topic describes how to select an experiment type. This procedure only supports quantitative experiments.

Procedure To select an experiment:

1. In the **Qualitative/Quantitative** view, select the **4 parameter logistic** fitting equation for all analytes.
2. Click the **Uniform fitting equations** checkbox.
3. Click **Next** to display the next view.

Specifying standards and analyte concentrations

About this topic This topic describes how to specify the number, units, and concentration levels for standards and analytes.

- Procedure**
- To specify the standards and analyte concentrations:
1. In the **Standards** view, specify the following for each standard:
 - The number of standards in the experiment (default = 10)
 - Units (pg/mL)
 - The concentration level (CC)

Std01 should be 0 pg/mL and Std10 should be the Top Standard, as defined in Table 1 of the BD CBA kit manual.

If your kit has varying assay ranges for each analyte, clear the **Uniform concentrations for all analytes** checkbox to assign concentrations to each analyte separately.
 2. Skip the next two steps in the Experiment Wizard. Click **Next** until the **Plate Layout Options** view appears.
-

Specifying the plate layout

About this topic This topic describes how to specify the layout of the 96-well filter plate.

Procedure

To specify the layout of the plate:

1. Select the **Place samples at the end** checkbox to facilitate more rapid file assignment. The plate layout (rows, columns, and blanks) does not need to match the plate layout from the experiment.
 2. Click **Next** to display the next view.
-

Naming the experiment

About this topic This topic describes how to name the experiment.

Procedure

To name the experiment:

1. Enter a name in the **Experiment Name** field.
2. Click **Finish**. Save the experiment to the default location.

Note: You can save the plex before you click **Finish**. To save the plex, select the **Save plex** checkbox and enter a name in the **Plex name** field. A saved plex includes all information about the experiment except for the number of samples and dilution factors. Saving plexes can greatly reduce the amount of data entry for subsequent analyses.

3. The **Plate Layout** workspace appears. Do not modify anything on this screen.
-

Associating samples with data files

About this topic This topic describes how to associate samples with data files.

Procedure

To associate samples with data files:

1. In the **Experiment** window, click the **File Assignment** tab.
2. Enter the instrument name and serial number the appropriate fields.
3. Assign the data files to the corresponding labels in the experiment.
 - a. In the left pane, select the first sample.
 - b. In the **Data files** column in the right pane, select the corresponding data file name (eg, Std01 should correspond to the data file that represents the 0 pg/mL standard).
4. Click the left arrow to assign the file.

When you select data in the left pane, the cursor automatically advances to the next sample.

5. Continue assigning files until all samples in the left pane are associated with data files.

If...	Then...
The samples in the left pane are in the same order as the files in the right pane	You can assign all files simultaneously by selecting the first sample and file, then clicking the double-left arrow button.

Starting analysis

About this topic This topic describes how to start analysis using the FCAP Array software.

Procedure To start analysis:

1. After you assign all files, click **Start analyzing this experiment** in the toolbar to begin analysis.



If...	Then...
The Start button is not green	<ul style="list-style-type: none"> • Make sure that the Instrument name and Serial number fields contain a value. • Make sure that all samples in the left pane have a file assigned to them.

2. A table of analysis messages appears. Review the messages, then click **OK**.
-

Printing, reviewing, and exporting analysis data

About this topic This topic describes how to print a report, review standard curves, and export raw data to a CSV file.

After analysis, three tabs (Report Printout, Standard Curves, and Raw Data) appear in the window.

Procedure

To print, review, or export analysis data:

- Click **Report Printout** to display and print a formatted report.
- Click **Standard Curves** to review the standard curves.
- Click **Raw Data** to display the experimental data in tabular form and export data in CSV (comma-separated value) format.

More information

See the *FCAP Array Software User's Guide* for details about saving and exporting experiment reports, or detailed descriptions of any of the software features.

United States

877.232.8995

Canada

888.259.0187

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