BD Cytometric Bead Array (CBA) Kits for BD Digital Flow Cytometers

Instruction Manual

BD

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History

Revision	Date	Change Made
645070	10/08	New document.

BD flow cytometers are class I (1) laser products
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Introduction

This section includes these topics:

- Using BD Digital Cytometers for BD CBA assays (page 6)
- Protocol workflow (page 7)

Using BD Digital Cytometers for BD CBA assays

About this topic

This topic describes the digital cytometers you can use with this CBA kit and describes the protocol and required materials.

Applicable cytometers

The cytometer setup information is for use only with the BD™ LSR II, BD FACSCanto™ II and BD FACSAria™ II flow cytometers using two lasers: 488 nm and 635 nm (BD LSR II) or 633 nm (BD FACSCanto II and BD FACSAria II).

BD FACSDiva™ software is required for acquiring samples and formatting data for subsequent analysis using FCAP Array™ Software.

About this protocol

The protocol described in this guide is intended for:

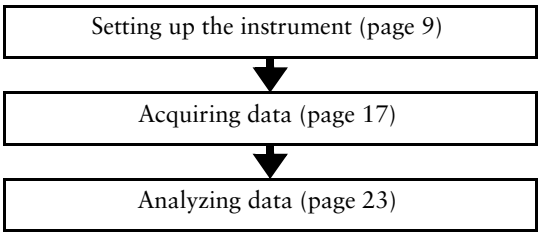
- Preparing cytometer setup beads.
- Setting up your cytometer using BD FACSDiva software.
- Acquiring data.
- Analyzing data with FCAP Array software.

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 instruction manual	Included in your BD CBA kit.
BD Digital Flow Cytometer sample experiment	Download from bdbiosciences.com/cbatemplates.

Protocol workflow



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Setting up the instrument

This section includes these topics:

- Instrument setup (page 10)
- Step 1: Preparing the template (page 11)
- Step 2: Preparing cytometer setup beads (page 12)
- Step 3: Creating a new experiment (page 12)

Instrument setup

About this topic

This topic describes the instrument setup procedure.

When to set up the instrument

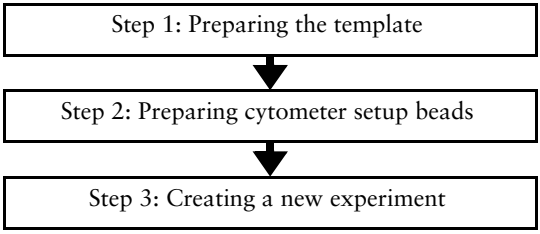
Perform instrument setup for each type of BD CBA Kit you plan to run (Human Th1/Th2, Mouse Inflammation, etc). Use the setup beads that are included with the CBA kit.

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

Before you begin

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

Procedure overview



Step 1: 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. In BD FACSDiva software, unzip the template and copy it into the templates folder.
2. In Windows® Explorer, navigate to C:\Program Files\BD FACSDiva\Templates.
3. Save a copy of the unzipped folder containing the BD CBA single color setup template in the Templates folder.

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

Step 2: Preparing cytometer setup beads

About this topic

This topic describes how to prepare the cytometer setup beads.

About the bead vials

You do not need to use the setup beads from vials B and C in this kit. These beads are used for setting up analog instruments.

Procedure

To prepare cytometer setup beads:

1. Add 100 µL of cytometer setup beads (vial D) to a cytometer setup tube.
2. Add 400 µL of wash buffer.

Step 3: Creating a new experiment

About this topic

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

Procedure

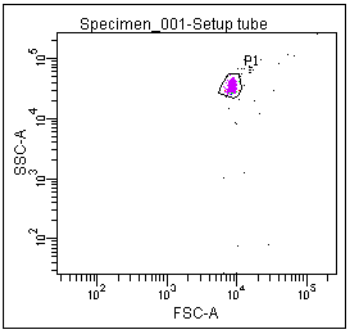
To create an experiment for instrument setup:

1. Start BD FACSDiva software.
2. Import the BD Digital Flow Cytometer sample experiment from bdbiosciences.com\cbatemplates.

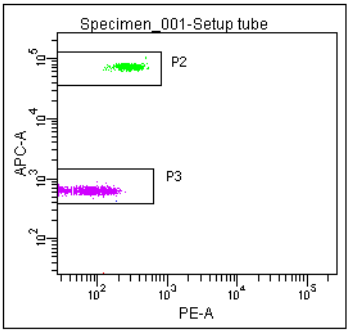
   Click on the **Sample Data** tab, then click the BD Digital Flow Cytometer sample experiment at the bottom of the page.
3. Open the imported experiment.
4. In the **Worksheet** frame, click the **CBA Instrument Setup Template** tab.

5. Make sure the Acquisition pointer is on the CBA instrument setup tube (in the Browser).
6. Begin with the instrument settings that are included in the sample experiment.

   You need to adjust instrument settings for each instrument.
7. Check the instrument laser delay for the 635 nm (BD LSR II) or 633 nm (BD FACSCanto II and BD FACSAria II) laser after opening the experiment to ensure that it is the correct setting for your instrument.
8. Load the cytometer setup beads tube.
9. Adjust the following:
   - FSC and SSC voltages so that the singlet bead population is in P1.
   - APC voltage until the mean of P2 is approximately 70,000.



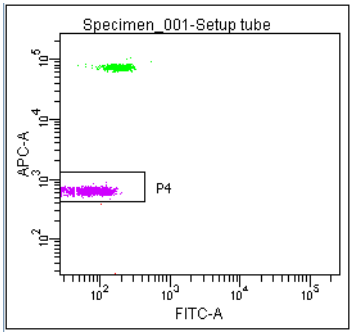
10. Adjust the PE voltage until the mean of P3 is approximately 70—75.



Experiment Name: Digital Instrument CBA Setup
Specimen Name: Specimen_001
Tube Name: Setup tube
Record Date: Jul 28, 2008 1:49:56 PM

Population	PE-A Mean	APC-A Mean
P2	290	73,793
P3	76	631

11. Adjust the FITC voltage until the mean of P4 is approximately 70—75.



Experiment Name:	Digital Instrument CBA Setup
Specimen Name:	Specimen_001
Tube Name:	Setup tube
Record Date:	Jul 28, 2008 1:49:56 PM

Population	FITC-A Mean
P4	73

12. Unload the tube.
13. Save the experiment.
14. Restart acquisition frequently during the instrument setup procedure to reset detected values after parameter adjustments.

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Acquiring data

This section includes these topics:

- Overview (page 18)
- Starting data acquisition (page 19)
- Exporting FCS data files from an experiment (page 19)
- Acquisition example (page 22)

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 tube 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. Select the CBA Acquisition template by clicking on the CBA acquisition global worksheet tab in the workspace.
2. Make sure the current tube pointer is set to the CBA tube. Generate new tubes until there is one for each standard and sample, renaming as appropriate.
3. Load the 0 pg/mL standard tube and, if necessary, adjust the gate to include only the singlet beads in the P1 gate.

   Samples are now ready to be acquired.

   For future CBA acquisitions, simply duplicate the CBA experiment without data and begin with Instrument Setup.

Starting data acquisition

About this topic

This topic describes how to use BD FACSDiva software to acquire data.

Before you begin

Run all standard tubes before you run any unknown test assay tubes.

Procedure

To start data acquisition:

1. Click **Acquire Data** in the **Acquisition Dashboard** to begin sample acquisition with the 0 pg/mL tube.
2. Continue increasing concentration through the 5,000 pg/mL tube.

Exporting FCS data files from an experiment

About this topic

You can export data from an experiment as FCS 2.0 or FCS 3.0 files if you want to analyze the data with different analysis software.

About exporting

- You can export data from closed or open experiments.
- You can select multiple items for simultaneous export.
- Individual FCS files are generated for each tube in a selected specimen or experiment.

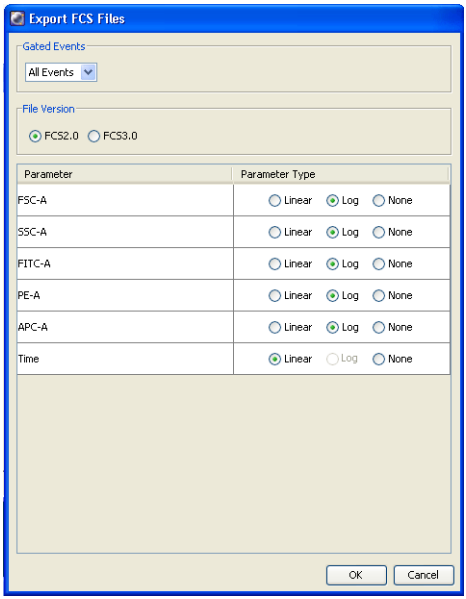
Procedure

To export data as FCS files:

1. Select the experiment, specimen, or tube(s) you want to export.

2. Select **File > Export > FCS files.**

   The **Export FCS Files** dialog appears.



3. Select **All Events** from the **Gated Events** menu.
4. Select **FCS 2.0** or **FCS 3.0** under **File Version.**
5. Select the following parameter types to export.

Parameter	Parameter type
FSC-A	Log
SSC-A	Log
FITC-A	Log
PE-A	Log
APC-A	Log

6. Select **None** for all other parameters in this list so that you do not export them.

FCAP Array software evaluates data for three parameters (SSC, PE, and APC).

7. Click **OK**.

   The **Save Export** dialog appears.

8. In the **Save Export** dialog, verify the file storage location or browse to the preferred storage location.

   a. Enter a different storage device name or folder name if you want to change the file storage location.

   b. Click **Details** to view the directory path and file name for each exported tube.

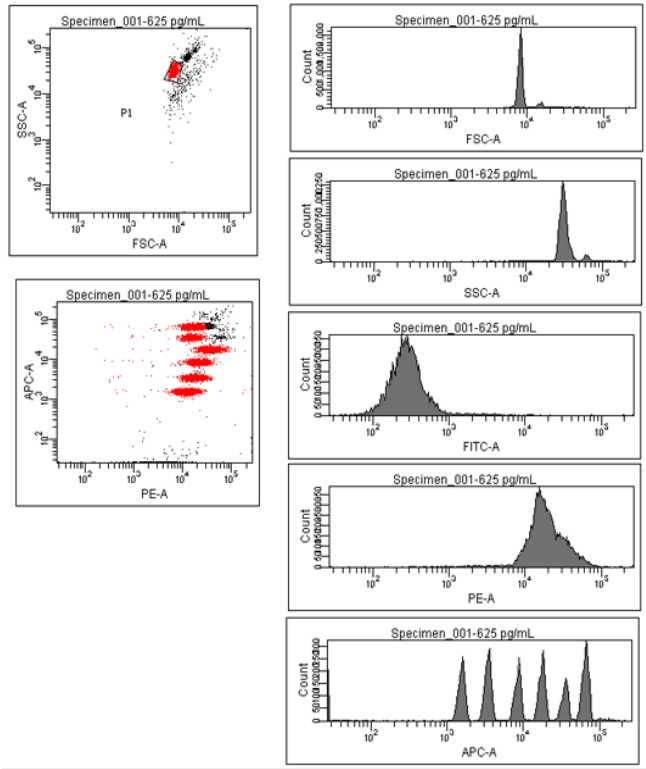
   The file names are the same as the tube names in the Browser and cannot be changed.

9. Click **Save** to export the files.

Acquisition example

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

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



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Analyzing data

This section includes these topics:

- Overview (page 24)
- Step 1: Creating a new experiment (page 26)
- Step 2: Creating and adding bead groups (page 27)
- Step 3: Defining clustering parameters (page 29)
- Step 4: Assigning analytes to bead populations (page 30)
- Step 5: Selecting an experiment type (page 30)
- Step 6: Specifying standards and concentrations (page 31)
- Step 7: Naming the experiment (page 32)
- Step 8: Assigning files to samples (page 33)
- Step 9: Associating samples with data files (page 34)
- Step 10: Starting analysis (page 36)
- Printing, reviewing, and exporting analysis data (page 37)

Overview

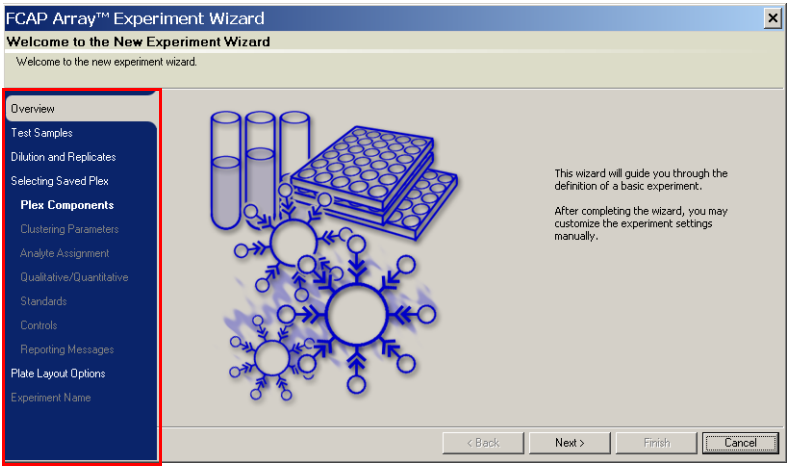
About analyzing data

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

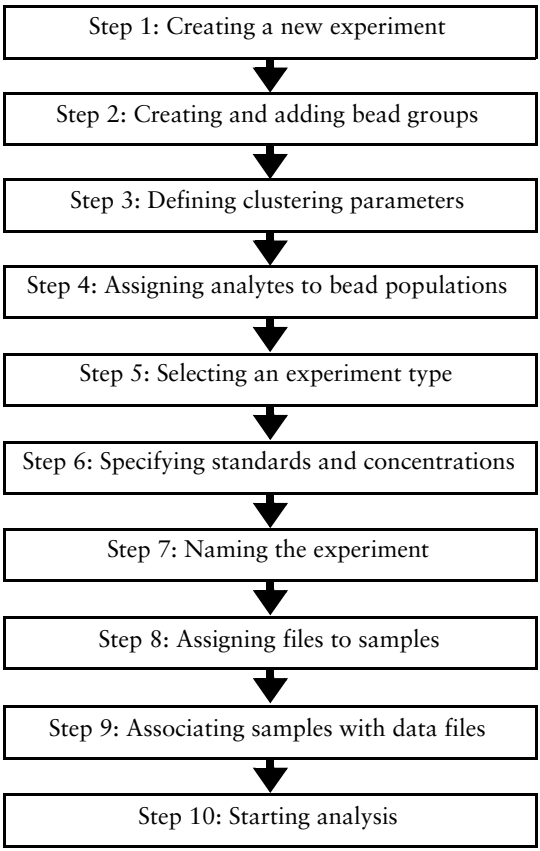
About the Experiment Wizard

The analysis steps described in the following procedure overview use the **FCAP Array Experiment Wizard**.

The wizard includes a list of experiment options in the left pane that you can select to display specific sets of tools or "views."



Procedure overview



Step 1: Creating a new experiment

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**.

Step 2: Creating and adding bead groups

About this topic

This topic describes how to create bead groups and add them to the library using the **FCAP Array Experiment Wizard**.

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 (dimmest 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.

Step 3: Defining clustering parameters

About this topic

This topic describes how to define clustering parameters using the **FCAP Array Experiment Wizard**.

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 FACSArray
   - 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.

Step 4: Assigning analytes to bead populations

About this topic

This topic describes how to assign analytes to bead populations using the **FCAP Array Experiment Wizard**.

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.

Step 5: Selecting an experiment type

About this topic

This topic describes how to select an experiment type using the **FCAP Array Experiment Wizard**. 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.

Step 6: Specifying standards and concentrations

About this topic

This topic describes how to specify the number, units, and concentration levels for standards and analytes using the **FCAP Array Experiment Wizard**.

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.

   Do not modify anything in this view.

Step 7: Naming the experiment

About this topic

This topic describes how to name the experiment using the **FCAP Array Experiment Wizard**.

Procedure

To name the experiment:

1. In the **Experiment Name** view, enter a name in the **Experiment Name** field.
2. (Optional) Select the **Save plex** checkbox and enter a name in the **Plex name** field.

   Saving plexes can greatly reduce the amount of data entry for subsequent analyses. A saved plex includes all information about the experiment except for the number of samples and dilution factors.
3. Click **Finish** to save the experiment in the default location and close the **FCAP Array Experiment Wizard**.

Step 8: Assigning files to samples

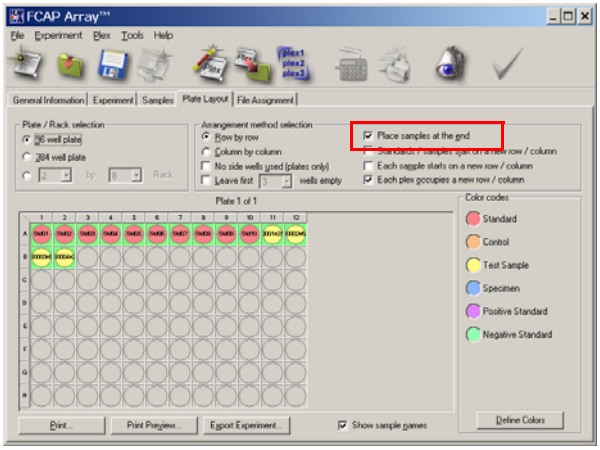
About this topic

This topic describes how to place samples at the end to facilitate more rapid file assignment using the main **FCAP Array Experiment** window.

Procedure

To assign files to tubes:

In the **Experiment** window, select the **Place samples at the end** checkbox.



Step 9: Associating samples with data files

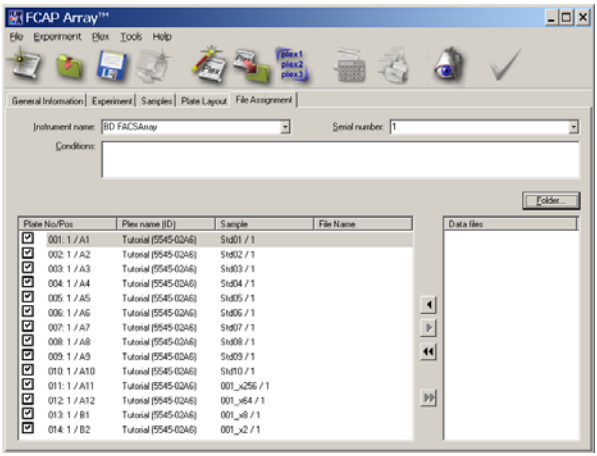
About this topic

This topic describes how to associate samples with data files using the main **FCAP Array Experiment** window.

Procedure

To associate samples with data files:

1. In the **Experiment** window, select the **File Assignment** tab.



2. Type 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.

Step 10: Starting analysis

About this

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

Procedure

To start analysis:

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

If...	Then...
The Start button is not green	• 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 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.

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