

FCAP Array™ Software Version 3.0 User's Guide

For Research Use Only

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Regulatory information

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

History

Revision	Date	Change made
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Introduction

This chapter includes the following topics:

- [About the documentation \(page 8\)](#)
- [Technical assistance \(page 9\)](#)
- [Limitations \(page 10\)](#)
- [System requirements \(page 11\)](#)
- [Basic terms \(page 12\)](#)

About the documentation

Introduction This topic describes the contents of the guide.

Documentation contents The *FCAP Array Software User's Guide* describes how to install and operate Flow Cytometric Analysis Program (FCAP) Array software. The software facilitates data analysis for multiplex bead assays.

This guide assumes you have a working knowledge of the basic Microsoft® Windows® operating system. If you are not familiar with the operating system of your computer, see the documentation provided with your system.

ReadMe file Before using FCAP Array™ software, review the ReadMe file by clicking the link in the Start menu. The ReadMe file contains important information not covered in this user's guide.

More information

- [Technical assistance \(page 9\)](#)
- [Limitations \(page 10\)](#)

Technical assistance

Introduction

This topic describes how to obtain technical assistance.

If you require technical assistance, contact your local BD Biosciences technical support representative or supplier.

Contact information

When contacting BD Biosciences, have the following information available:

- Version of FCAP Array software you are using.
- Name and version of the acquisition software you are using.
- Any error messages.
- Details of recent cytometer system performance.

For FCAP Array software support from within the US and Canada, call 877.232.8995.

Customers outside the US and Canada, contact your local BD representative or distributor.

More information

- [Troubleshooting \(page 116\)](#)
-

Limitations

Introduction	This topic describes the limitations for use of the software.
Limitations	For Research Use Only. Not for use in diagnostic or therapeutic procedures.
Compatibility with previous versions	<p>FCAP Array version 3.0 cannot use experiment, plex, or bead files from FCAP Array version 1.0.</p> <p>The security key for version 1.0 does not work with version 3.0. Therefore, if you want to run both versions on the same computer, you must insert both security keys in the computer.</p> <p>The gating and curve fitting algorithms for previous versions of FCAP Array software are different. The user can expect bead clusters and standard curves to vary slightly if comparing version 3.0 to a previous version.</p>
More information	<ul style="list-style-type: none">• Technical assistance (page 9)• System requirements (page 11)

System requirements

Introduction

This topic specifies the system requirements for using FCAP Array software.

Minimum PC configuration

FCAP Array software requires a computer with the following minimum configuration:

Item	Specification
CPU	Intel Pentium® 4 (2 GHz or equivalent)
RAM	512 MB or higher
Video RAM	16 MB or higher
Hard drive space	50 MB for installation
Monitor resolution	XGA, 1024 x 768 pixels or higher (1280 x 1024 recommended)
Monitor color	16-bit color or higher
DVD-ROM drive	Required for software installation
USB port	Required for security key

Software requirements

FCAP Array software requires the following:

- Microsoft Windows XP or Windows 7 (32-bit and 64-bit versions)
- Microsoft .NET 3.5 framework
- Microsoft SQL Server Compact Edition 3.5

The installer will install .NET framework and SQL Server if they are not already on the computer.

-
- More information**
- [Technical assistance \(page 9\)](#)
 - [Limitations \(page 10\)](#)
-

Basic terms

Introduction This topic describes the basic terms used throughout the manual to indicate specific constructs, procedures, or concepts as they apply to FCAP Array software.

Terms The basic terms used throughout this user guide are defined in the following table.

Term	Definition
bead	<ul style="list-style-type: none"> • An analyte-specific capture particle with distinctive, discrete fluorescence characteristics. All instances of an individual bead bind to the same analyte and have the same fluorescence characteristics. • An FCAP Array software construct that specifies the analyte binding and fluorescence characteristics of a bead.
bead assay	A method of using beads and reporter antibodies to detect or measure the concentration of analytes in a sample.
cluster	A population of events in a bead assay data file. Each cluster corresponds to a bead population. Therefore, the events in a cluster have distinctive, discrete fluorescence characteristics.
experiment	<ul style="list-style-type: none"> • A bead assay. • An FCAP Array software construct that defines the plex(es), standards, controls, and test samples for a bead assay.

Term	Definition
plex	<ul style="list-style-type: none">• One or more beads used in a bead assay.• An FCAP Array software construct that specifies a set of standards, beads, analysis models, instrument settings, and standard curves.
replicate(s)	One or more copies of a sample. When more than one replicate of a sample is used, the replicate results are averaged to determine the result reported for the sample.
sample	A solution containing analytes to be measured by a bead assay. A sample can be a test, a standard, or a control.

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Installing and starting the software

This chapter covers the following topics:

- [Installing FCAP Array software \(page 16\)](#)
- [Starting the software \(page 17\)](#)
- [Obtaining the bead library file \(page 19\)](#)
- [Uninstalling FCAP Array software \(page 20\)](#)

Installing FCAP Array software

Introduction This topic provides instructions for installing FCAP Array software version 3.0.

Caution



Caution! Do not install the FCAP Array version 3.0 security key until after step 6 in the procedure. The software and drivers must be installed first.

FCAP Array version 3.0 can exist concurrently with FCAP Array version 1.0 on the same PC. See [Limitations \(page 10\)](#) for more information.

Procedure

To install FCAP Array software:

1. Ensure that no other programs are running.
2. Insert the FCAP Array software installation disc into the DVD-ROM drive.

If the installer does not start up automatically, use Windows Explorer to view the disc contents, then double-click the **Setup.exe** icon.

3. Click **Next** on the installer **Welcome** screen.
4. Read the license information, then select **I Agree** and click **Next**.
5. Note the destination folder for FCAP Array software and click **Install**.

We recommend that you accept the default location.

6. Click **Finish** to exit the installer.
 7. Install the FCAP Array security key in a USB port.
-

Next step

Proceed to [Starting the software \(page 17\)](#).

More information

- [System requirements \(page 11\)](#)
 - [Starting the software \(page 17\)](#)
 - [Uninstalling FCAP Array software \(page 20\)](#)
-

Starting the software

Introduction

This topic describes how to start the software.

Procedure**To start the software:**

1. Double-click the FCAP Array V3.0 desktop icon.

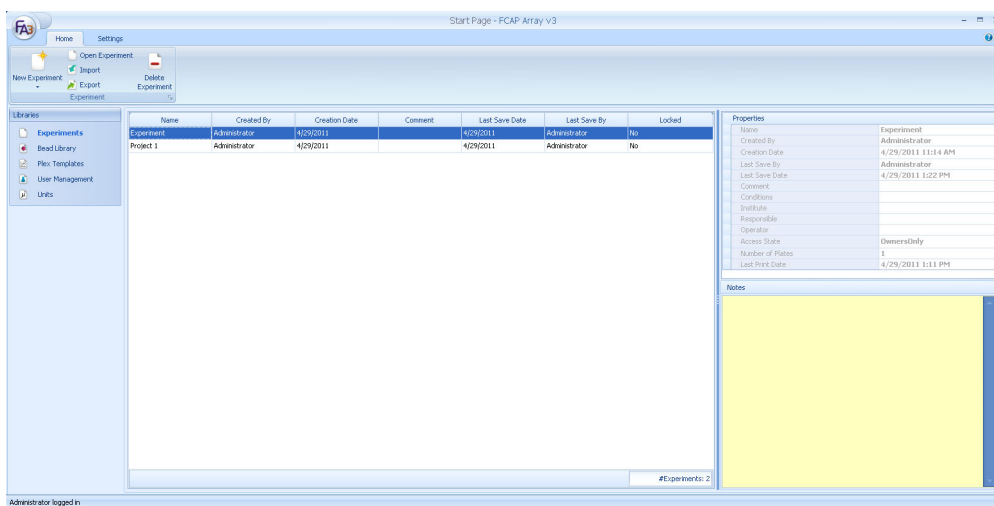
As the software starts, the **FCAP Array Login** dialog opens.

2. Type your user name and password.

User names and passwords are case-sensitive. The password can be blank. When FCAP Array software is installed there is a default user with the user name *Administrator*, password *welcome*, and administrative rights. The *Administrator* password should be changed immediately, and accounts created as needed.

3. Click **Login**.

As the software starts, the main window opens with the experiments library displayed.



Starting from BD FACSuite software

When FCAP Array and BD FACSuite™ software are installed on the same computer, you can start FCAP Array from the menu bar in BD FACSuite software.

See the *BD FACSuite™ System Reference* for setup instructions.

Next step

Proceed to [Obtaining the bead library file \(page 19\)](#).

More information

- [New experiment overview \(page 28\)](#)
- [BD FACSuite workflow overview \(page 94\)](#)

Obtaining the bead library file

Introduction

This topic describes how to obtain the bead library file for FCAP Array software. This file is used to populate the bead library with information about BD CBA reagents.

Note that the bead file for FCAP Array software version 3.0 has a different format than for version 1.0.

Procedure

To download and import the bead library file:

1. Navigate to bdbiosciences.com/cbasetup.
 2. Download the bead library file for FCAP Array software version 3.0.
 3. After the download is complete, unzip the file.
 4. Click the **Bead Library** icon.
 5. Click the **Import** icon.
The **Import** dialog opens.
 6. Select the downloaded XML file.
 7. Click **Open**.
-

More information

- [Working with libraries \(page 100\)](#)
-

Uninstalling FCAP Array software

Introduction This topic describes how to uninstall FCAP Array software.

Procedure To uninstall FCAP Array software:

1. Select Start > Programs > FCAP Array V3 > Uninstall.

More information

- [System requirements \(page 11\)](#)
- [Limitations \(page 10\)](#)
- [Installing FCAP Array software \(page 16\)](#)

3

Overview

This chapter covers the following topics:

- [Software overview \(page 22\)](#)
- [Workflow overview \(page 24\)](#)

Software overview

Introduction This topic gives an overview of FCAP Array software version 3.0 and describes the quantitative, qualitative, and quality control analysis methods.

Overview FCAP Array software facilitates data analysis of bead assays. These assays can detect the presence of, or determine concentrations for, multiple analytes (for example, proteins and peptides) in a sample.

Samples are acquired with a flow cytometer and acquisition software capable of saving data in flow cytometry standard (FCS) 2.0 or 3.0 file format. FCAP Array software analyzes the FCS files to find bead populations by clustering. Reporter antibody fluorescence within a population measures analyte-specific binding.

Quantitative analysis Quantitative analysis determines analyte concentrations based on the known concentration values of a set of standards. FCAP Array software can determine analyte concentration for many analytes (beads) per sample. The number of concentration levels used to calibrate the standard curve and the number of sample and standard replicates are easily adjusted.

FCAP Array software reads the FCS data files, locates clusters (to which analytes have been assigned), and then determines the MFI of the detector antibody for each analyte. From the MFI of the standards, the software calculates a curve that describes the relationship between MFI and concentration. The curve used to fit the data is selected from several mathematical models. A standard curve is used to calculate the concentration values for each of the measured analytes in a sample.

Qualitative analysis Qualitative analysis determines whether a specific type of analyte exists in a sample based on known controls. FCAP Array software can determine analyte existence for many analytes (beads) per sample. Experiments can include positive controls, negative controls, or both.

FCAP Array software reads the FCS data files from an experiment, locates clusters (to which analytes have been assigned), and then determines the MFI of the detector antibody for each analyte. The control data is used to determine MFI cutoff values for each analyte. Test sample results are based on the cutoff values.

Quality control analysis

For beads that have two reporter parameters, the quality control analysis model is used. FCAP Array software compares the MFI of a sample to a user-defined range or a threshold determined from a control. Values that are out of range are flagged in the results and reports.

Quality control analysis can be customized to calculate the MFI for a negative standard by multiplying that MFI by a user-defined multiplier. This calculated cut-off value is used to determine whether a specific analyte or analytes exist in a sample.

What's new in version 3.0

FCAP Array version 3.0 has the following new features.

- Support of FCS 3.0 files
- The ability to utilize CBA keywords from BD FACSuite software to simplify your workflow
- A standard dilution calculator
- Debris filtering and manual clustering tools
- Graphical reporting of results
- Exporting of reports in multiple formats, including PDF

More information

- [Limitations \(page 10\)](#)
 - [System requirements \(page 11\)](#)
-

Workflow overview

Introduction

This topic describes the two workflows for using FCAP Array software.

- **Standard workflow.** Analyzing FCS files from standard cytometry software, including BD CellQuest™ Pro software, BD FACSAArray™ software, and BD FACSDiva™ software.
 - **BD FACSuite workflow.** Analyzing FCS files from BD FACSuite software for an optimized workflow.
-

Standard workflow description

The standard workflow is used when analyzing FCS files from most cytometry software applications. You design an experiment in FCAP Array software that reflects the data contained in the FCS files that you are importing. The process of designing this experiment can be greatly simplified if you first design a plex, which defines beads, standards, analysis models, and instrument settings that are common to multiple experiments.

After designing the experiment, you proceed to analyze the FCS files. The results are calculated and displayed in the same manner for both the standard workflow and BD FACSuite workflow.

Standard workflow stages

The standard workflow includes these stages.

Stage	Description
1	Starting the software (page 17)
2	Creating a new experiment (page 27)
3	Working with plexes (page 47)
4	Results and reports management (page 79)

**BD FACSuite
workflow
description**

The BD FACSuite workflow is optimized for BD FACSuite software, but you can use the standard workflow as well. When you use BD FACSuite software to set up an experiment and run an acquisition, you add CBA-specific keywords which will be included in the exported FCS files.

FCAP Array software reads the FCS files and creates a new experiment based on the keywords. The workflow is streamlined and you are able to view analysis results quickly.

**BD FACSuite
workflow stages**

The BD FACSuite workflow includes these stages.

Stage	Description
1	Starting the software (page 17)
2	Creating a new experiment from BD FACSuite files (page 96)
3	Evaluating the experiment (page 98)
4	Results and reports management (page 79)

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4

Creating a new experiment

This chapter covers the following topics:

- [New experiment overview \(page 28\)](#)
- [Creating a new experiment \(page 29\)](#)
- [Adding plexes \(page 31\)](#)
- [Setting the sample layout \(page 33\)](#)
- [Adding samples \(page 35\)](#)
- [Modifying samples in the layout view \(page 40\)](#)
- [Working with experiment information \(page 43\)](#)

New experiment overview

Introduction

This topic describes the workflow for creating a new experiment. You can use this workflow for analyzing data from multiple flow cytometry systems. If you use BD FACSuite software, you can use either this workflow or the BD FACSuite workflow, which provides better optimization.

New experiment workflow

The following table shows the stages in a typical new experiment workflow.

Stage	Description
1	Creating a new experiment (page 29)
2	Adding plexes (page 31)
3	Setting the sample layout (page 33)
4	Adding samples (page 35)
5	Working with experiment information (page 43)

More information

- [Workflow overview \(page 24\)](#)
 - [Basic terms \(page 12\)](#)
 - [Working with plexes \(page 47\)](#)
 - [Modifying samples in the layout view \(page 40\)](#)
-

Creating a new experiment

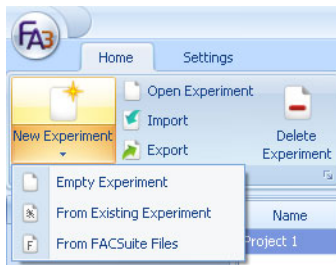
Introduction

This topic describes how to create a new experiment.

Procedure

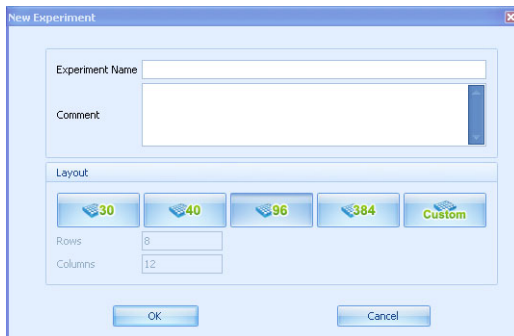
To create a new experiment:

1. Click the lower half of the **New Experiment** button on the application toolbar and select one of the following:
 - **Empty Experiment.** Select this option to create a new experiment.
 - **From Existing Experiment.** Select this option to use a saved experiment.
 - **From FACSuite Files.** Select this option only if you want to use the BD FACSuite workflow. See [Using the BD FACSuite workflow \(page 93\)](#).



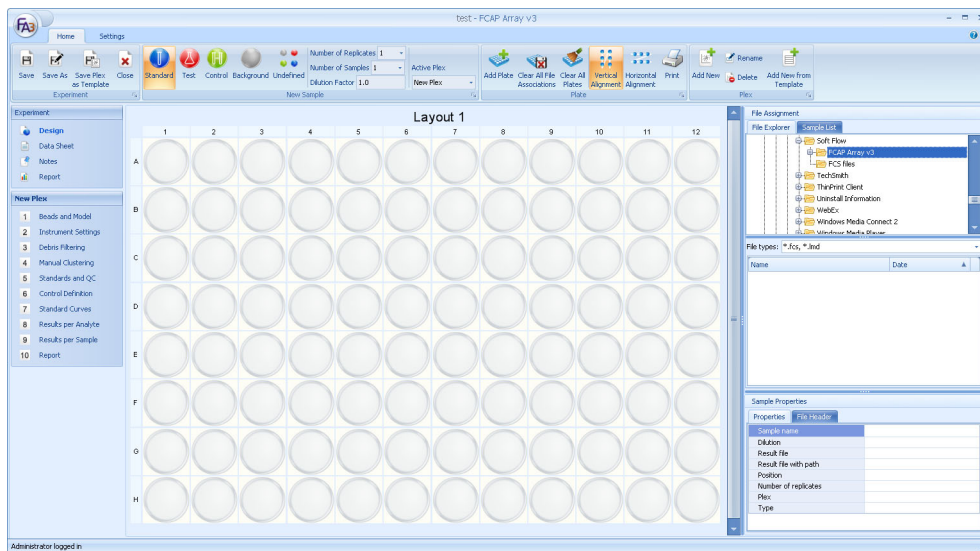
- If you have plex template files in the plex template library, you can create a new experiment from that location. Select a template, then click the **New Experiment from Template** icon.
2. Enter a name and an optional comment in the **New Experiment** dialog.
 3. Select a size for the sample layout.

The default is a 96-well plate. Also, use this layout if samples are acquired manually in tubes.



4. Click **OK**.

The design view opens.



Next step

Proceed to [Adding plexes \(page 31\)](#).

More information

- [Workflow overview \(page 24\)](#)
- [New experiment overview \(page 28\)](#)

Adding plexes

Introduction

This topic describes how to add plexes.

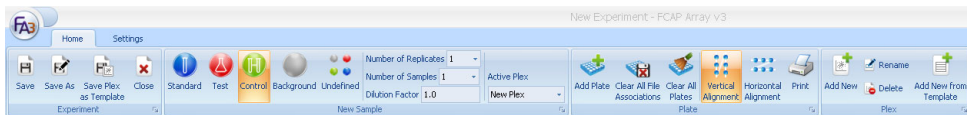
About new and saved plexes

An experiment always contains one plex as the default setting, but you can have multiple plexes in an experiment. By default, a plex named New Plex is added to a new experiment.

Saving a plex as a template streamlines your workflow by creating a flexible template which does not have restrictions on required content. Using a saved template reduces manual data entry in subsequent experiments.

See [Plex overview \(page 48\)](#).

The plex functions are accessed from the groups of buttons on the ribbon as shown in the following figure.

**Adding a new plex** To add a new plex:

1. Click **Add New** in the **Plex** group.

The **Add New Plex** dialog opens.

2. Enter a name for the plex and click **OK**.

The new plex will be the active one in the experiment.

Adding a new plex from a template

To add a new plex from a template:

1. Click **Add New from Template** in the **Plex** group.
The **Add New Plex from Template** dialog opens.
2. From the **Plex Template** menu, select a template from the library.
3. In the **New Plex Name** field, enter a name, then click **OK**.

The new plex will be the active one in the experiment.

Changing the active plex

To change the active plex:

1. Click a plex from the **Active Plex** list.

The selected plex becomes the active one in the experiment. New samples are added to the active plex. Samples belonging to other plexes appear in a pale shade.

Options for the active plex are shown in the navigation panel on the left.

Deleting the active plex

To delete the active plex:

1. Click **Delete** in the **Plex** group.

The **Confirm plex deletion** dialog opens.

2. Click **Yes** to confirm.
-

Saving the active plex as a template

To save the active plex as a template:

1. Click **Save Plex as Template** in the **Experiment** group.

The **Save Plex Template** dialog opens.

2. Enter a name for the plex template in the **Plex Template Name** field and click **OK**.
-

Next step

Proceed to [Setting the sample layout \(page 33\)](#).

More information

- [Workflow overview \(page 24\)](#)
 - [New experiment overview \(page 28\)](#)
 - [Creating a new experiment \(page 29\)](#)
 - [Working with plexes \(page 47\)](#)
-

Setting the sample layout

Introduction

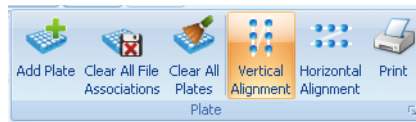
This topic describes how to set up the sample layout in an experiment.

Note that when samples have been acquired manually in tubes, they still need to be arranged on a plate for use in the software.

Working with plates

An experiment always contains one plate as the default setting, but you can have multiple plates in an experiment. The controls for working with plates are located in the **Plate** group on the ribbon.

You can also right-click in a plate to open a menu to perform some of the functions.



Adding a plate**To add a plate:**

1. Click **Add Plate** in the **Plate** group.

The new plate is added below the current plate. If you cannot see the plate, scroll down and make sure the new plate was added.

Changing the plate alignment

The alignment direction for the samples on a plate is set to horizontal by default. When selecting a direction, make sure it matches your acquisition order. The selected direction determines the order when adding multiple samples or assigning multiple files in one motion. File assignment should match the order and the direction of added samples.

The selected direction also influences the order of samples in the following screens: debris filtering, manual clustering, standards and QC, results per analyte, results per sample, and report.

To change the plate alignment:

1. Click the **Vertical Alignment** or **Horizontal Alignment** icons in the **Plate** group.
-

Removing a plate**To remove a plate:**

1. Right-click a plate and select **Remove Plate**.

The selected plate and all samples within the selected plate are removed from the experiment.

Clearing a plate**To clear a plate:**

1. Right-click a plate and select **Clear Plate**.

All samples are removed from the selected plate in the experiment.

Clearing all plates**To clear all plates:**

1. Right-click a plate and select **Clear All Plates**.

All samples are removed from all plates in the experiment.

Next step

Proceed to [Adding samples \(page 35\)](#).

More information

- [Workflow overview \(page 24\)](#)
 - [New experiment overview \(page 28\)](#)
 - [Adding plexes \(page 31\)](#)
-

Adding samples

Introduction

This topic describes how to add samples to a plate.

About sample types

There are five different sample types: Standard, Test, Control, Background, and Undefined. The control and background sample types are not used in BD CBA assays.

Note that a sample type is always selected and that clicking the sample layout creates the highlighted sample type.

Each type has a different color and icon in the ribbon.



Methods for adding samples

There are three methods for adding samples.

- **Adding samples by designing the layout.** With this method you add samples first and then assign FCS files to the selected samples. Use this method when adding samples with replicates.
- **Adding files with sample types.** With this method you select the sample type and the corresponding FCS files from the **File Explorer** window and add them to the plate. When the files are in the correct order in the **File Explorer**, the assignment of files to the plate can be done in one step.
- **Adding undefined samples.** With this method you add all files to the experiment and then define the sample types one by one.

Standards are arranged for the standard curve in alphabetical order by sample name (Std001, Std002, Std003, etc). Confirm that names are assigned in such a way that standards will be plotted in the correct order.

Wells containing assigned sample files have a floppy disc icon.



Adding samples by designing the layout

To add samples by designing the layout:

1. Select one of the four sample types (Undefined is not applicable with this method) from the **New Sample** group by clicking the icon.
2. Define the number of samples, number of replicates, and the dilution factor.

3. Select the first well you want to start with and click it.

According to your settings, the sample type icon and the number of replicates appear on the plate (number of replicates multiplied by number of samples). If the number of replicates exceeds the available wells, then a new plate is automatically added to the experiment.

4. Select FCS files from the **File Assignment** panel and drag and drop them onto the plate.

For more detailed instructions on selecting FCS files, see [Selecting FCS files \(page 39\)](#).

Adding files with sample types

To add files with sample types:

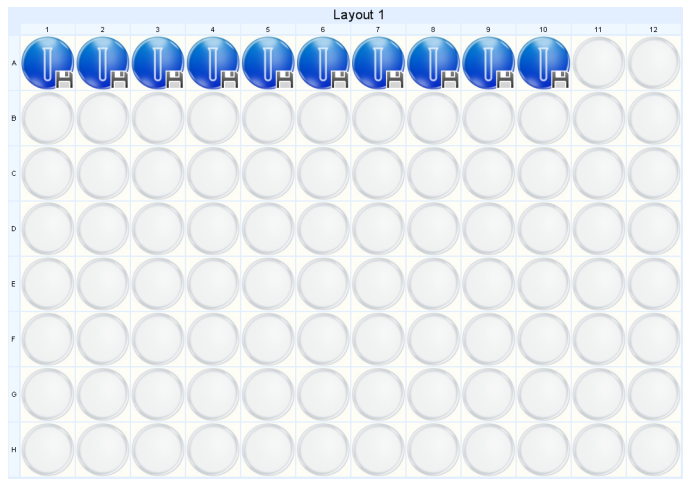
1. Click one of the four sample type icons (except Undefined).
2. If test samples were diluted, define the **Dilution Factor**.

It is not necessary to define dilution factors for standards.

3. Select FCS files from the **File Assignment** panel and drag and drop them onto the plate. When your files are in the correct order in the **File Explorer**, you can assign your files in one motion.

For more detailed instructions on selecting FCS files, see [Selecting FCS files \(page 39\)](#).

The following figure shows a typical plate layout at this stage.



Adding undefined samples

To add undefined samples:

1. Click the **Undefined** sample type icon in the **New Sample** group.
2. Define the **Dilution Factor**, if necessary.
3. Select FCS files from the **File Assignment** panel and drag and drop them onto the plate.

For more detailed instructions on selecting FCS files, see [Selecting FCS files \(page 39\)](#).

4. Define the sample types.
 - a. Select one of the four colored circles in the well.

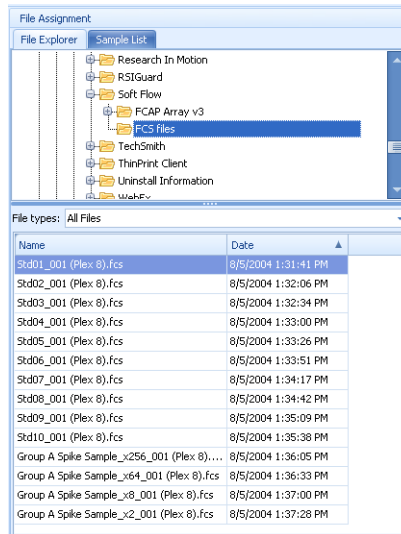


Selecting FCS files

To select FCS files:

1. Navigate to your FCS files from the **File Explorer** tab on the **File Assignment** panel.

The FCS files are listed in the lower window.



2. Filter by file type in the **File types** list.

If you do not see your files listed, change the **File types** to **All Files**. This displays files that do not have a .fcs or .lmd file extension.

3. Files are displayed in ascending order by acquisition date and time in the default settings. You can change the order for both name and date from ascending to descending by clicking the column header.
4. You can select multiple files by Ctrl + clicking the files. You can also select by Shift + clicking the first file while clicking the last file.

Next step

Proceed to [Working with experiment information \(page 43\)](#).

-
- More information**
- [Modifying samples in the layout view \(page 40\)](#)
 - [Setting the sample layout \(page 33\)](#)
 - [Workflow overview \(page 24\)](#)
 - [New experiment overview \(page 28\)](#)
-

Modifying samples in the layout view

Introduction This topic describes how to modify and move samples once they have been added to a plate.

Note that the well counters for the numbering of the sample name do not reset unless you delete all samples of a given type.

Selecting samples To select a sample:

1. Click on its well.

You can select multiple samples by Ctrl + clicking the desired wells or by drawing a rectangle around the desired wells. The background color of the wells indicates the selection. When removing samples, all selected wells become empty.

Moving samples To move samples:

1. Select one or more samples, then drag the samples to the new position.

Samples cannot be moved to a position on a different plate.

Changing the sample type To change the sample type:

1. Right-click the sample you want to change and select **Change Sample Type**.

The changed sample is now undefined.

2. Redefine the sample type by clicking the icon (shown in the selected well) of the sample type you wish to assign.

To change the sample type, the sample must have a file associated with it.

Removing a sample To remove a sample, do one of the following:

- Right-click the sample you want to remove and select **Remove Sample**.
- Click the well, then press the **Delete** key. When removing multiple samples, all selected wells become empty.

Clearing the file assignment

To clear the file association from a sample, do one of the following:

- Right-click a well and select **Remove file association**.
- Click the **Clear All File Associations** icon in the **Plate** group.
- Right-click a well and select **Clear all file associations**.

Viewing sample properties

To view sample properties, do one of the following:

- Click the **Properties** tab to view data about a selected sample.
- Click the **File Header** tab to see FCS file header keywords for a selected sample.

The **File Header** table is empty when no well is selected.

Sample Properties	
Properties File Header	
Sample name	Std001
Dilution	1
Result file	Std01_001 (Plex 8).fcs
Result file with path	C:\Documents and Settings\adetrann...
Position	1 - A1
Number of replicates	1
Plex	New Plex
Type	Standard

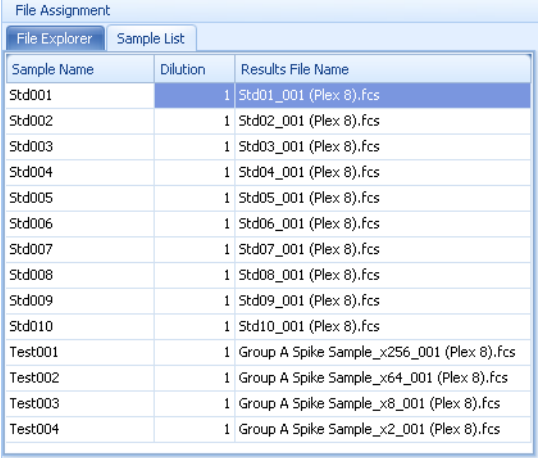
Modifying the sample name and dilution factor

Samples are named automatically with a default setting when they are imported. When using the standard workflow, the prefix for standards is *Std*, for test samples it is *Test*, for controls it is *Control* and for background it is *Background*. You can modify the name of the sample and its dilution factor.

To modify sample information using the Sample List tab:

1. Click the **Sample List** tab on the **File Assignment** panel.

Using the **Sample List** tab enables you to view all the samples at once and to enter data more rapidly.



Sample Name	Dilution	Results File Name
Std001	1	Std01_001 (Plex 8).fcs
Std002	1	Std02_001 (Plex 8).fcs
Std003	1	Std03_001 (Plex 8).fcs
Std004	1	Std04_001 (Plex 8).fcs
Std005	1	Std05_001 (Plex 8).fcs
Std006	1	Std06_001 (Plex 8).fcs
Std007	1	Std07_001 (Plex 8).fcs
Std008	1	Std08_001 (Plex 8).fcs
Std009	1	Std09_001 (Plex 8).fcs
Std010	1	Std10_001 (Plex 8).fcs
Test001	1	Group A Spike Sample_x256_001 (Plex 8).fcs
Test002	1	Group A Spike Sample_x64_001 (Plex 8).fcs
Test003	1	Group A Spike Sample_x8_001 (Plex 8).fcs
Test004	1	Group A Spike Sample_x2_001 (Plex 8).fcs

2. Select a sample by clicking in the **Sample List**.
3. Edit the name and press Enter.
4. Select a sample by clicking in the **Dilution** field.
5. Edit the value and press Enter.

Next step

Proceed to [Working with experiment information \(page 43\)](#).

More information

- [Adding samples \(page 35\)](#)
- [Workflow overview \(page 24\)](#)
- [New experiment overview \(page 28\)](#)

Working with experiment information

Introduction

This topic describes how to enter information about an experiment in the data sheet. You can also add or remove users from the list of experiment owners to change access rights to the experiment. You can also change the security rights for the experiment.

Entering information

To enter information in the experiment data sheet:

1. Click the **Data Sheet** icon in the **Experiment** panel.
2. Click in the fields next to each field type and enter information about the experiment.

Name	New Experiment
Comment	
Responsible	
Institute	
Operator	
Conditions	
Creation Date	5/3/2011 5:53 PM
Default Plate Size	8 x 12
Last Print Date	5/3/2011 5:58 PM
Last Save By	Administrator
Last Save Date	5/3/2011 5:58 PM
Created By	Administrator
Plate Count	1
Plate Direction	TopDown
Instrument Type	FACSAArray
Instrument Serial Number	1

Changing users in an experiment

You can add or remove users from an experiment in the **Experiment Owners** table. Users added to the **Experiment Owners** list can open, read, and modify the shared experiment.

To add a user:

1. Select a user name from the **All Users** list.

All Users					
Login Name	Full Name	EMail	Contact Info	Department	Is Admin
user1					No
user3					No
Administrator					Yes

Experiment Owners					
Login Name	Full Name	EMail	Contact Info	Department	Is Admin
Administrator					Yes
user2					No

2. Click **Add Selected User** in the **Security** group on the ribbon.

The selected user is added to the **Experiment Owners** list.

To remove a user:

1. Select the name from the **Experiment Owners** list.
2. Click the **Remove Selected User** icon in the **Security** group on the ribbon.

The selected user is removed from the **Experiment Owners** list.

Changing user security rights in an experiment

Note that a user with administrator rights can view and edit all experiments.

To change user security rights in an experiment, select one of the following:

- Click the **Public** icon in the **Security** group.
This enables editing of the experiment by all users.
- Click the **Owners Only** icon in the **Security** group.
This enables editing of the experiment only by the respective owner(s) and administrators.
- Click the **Locked** icon in the **Security** group.
This locks the experiment from any editing. Click the **Locked** icon again to allow editing.

Adding notes to an experiment

To add notes to an experiment:

1. Click the **Notes** icon in the **Experiment** panel.

The **Notes** dialog opens.

2. Type in your notes and click **OK**.

Your notes are shown when you select an experiment in the experiment library.

Next step

Proceed to [Working with plexes \(page 47\)](#).

More information

- [Workflow overview \(page 24\)](#)
 - [New experiment overview \(page 28\)](#)
 - [Modifying samples in the layout view \(page 40\)](#)
-

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5

Working with plexes

This chapter covers the following topics:

- [Plex overview \(page 48\)](#)
- [Selecting beads and analysis model \(page 51\)](#)
- [Selecting instrument settings \(page 53\)](#)
- [Assigning beads to clusters \(page 55\)](#)
- [Using debris filtering \(page 57\)](#)
- [Using manual clustering \(page 59\)](#)
- [Defining standards and QC \(page 64\)](#)
- [Defining controls \(page 72\)](#)
- [Viewing standard curves \(page 74\)](#)

Plex overview

Introduction This topic describes plexes, plex templates, and the stages in the workflow for creating a plex.

About plexes An FCAP Array experiment contains one or more plexes. A plex is created or modified within an experiment. Plexes can be saved to the plex templates library to be reused or exported to other users.

Plex navigation panel Creating a plex is typically done in the order shown in the **Plex** navigation panel as shown in the following figure.



Plex template

The plex template stores the following information.

Parameter	Content
Standard Samples	Number, name, concentration, number of replicates
Beads and Model	Selected beads, lot numbers, analysis models
Instrument Settings	Scatter and clustering parameters, cluster positions, bead-cluster assignment
Debris Filtering	Defined debris filtering gates, status of the filtering (active or not)
Standards and QC	Uniformity, concentrations, measurement units (quantitative model), positive/negative standard selection (qualitative model), MFI threshold values (QC model)
Standard Curves	Axis types (linear/logarithmic), force through zero, weighting type, fitting type, fitting accuracy (%)

The plex template is flexible. There are no restrictions regarding the information required to save a plex as a template.

Plex creation workflow

The workflow of creating a plex includes these stages.

Stage	Description
1	Selecting beads and analysis model (page 51)
2	Selecting instrument settings (page 53)
3	Assigning beads to clusters (page 55)
4	Using debris filtering (page 57)
5	Using manual clustering (page 59)
6	Defining standards and QC (page 64)
7	Defining controls (page 72)
8	Viewing standard curves (page 74)

More information

- [Workflow overview \(page 24\)](#)
 - [Adding plexes \(page 31\)](#)
-

Selecting beads and analysis model

Introduction

This topic describes the process of adding beads to a plex and selecting the analysis model.

Before you begin

If necessary, add beads to the bead library. When you first install FCAP Array, the bead library is empty. For BD CBA reagents, we recommend adding beads to the bead library by importing an XML file. See [Importing a bead group \(page 107\)](#).

Selecting beads for a plex

To select beads for a plex:

1. Click **Beads and Model** in the **Plex** navigation panel to define the beads needed for your plex.

The **Selected Beads** panel opens.

Selected Beads						
Bead Name	Lot Number	Catalog Number	Name	Analyte		
				Model	Quantitative	2nd Reporter
A5		11	Human IL-4	Quantitative	No	
A7		22	Human IL-6	Quantitative	No	
A8		33	Human IL-7	Quantitative	No	
B6		44	Human IL-8	Quantitative	No	
C4		55	Human Angiogenin	Quantitative	No	
D7		66	Human T-alpha	Quantitative	No	

Bead Group	Group Description
<input checked="" type="checkbox"/> All Beads	
<input type="checkbox"/> Human Soluble Protein	BD CBA Flex Sets
<input type="checkbox"/> Mouse Th1/Th2 Cytokine Kit - 551267	BD CBA Kit
<input type="checkbox"/> Cell Signaling	BD CBA Flex Sets
<input type="checkbox"/> Human Enhanced Sensitivity (ES)	BD CBA Flex Sets
<input type="checkbox"/> Human Immunoglobulin	BD CBA Flex Sets
<input type="checkbox"/> Mouse Soluble Protein	BD CBA Flex Sets
<input type="checkbox"/> Rat Soluble Protein	BD CBA Flex Sets
<input type="checkbox"/> Human Anaphylatoxin Kit - 561418	BD CBA Kit
<input type="checkbox"/> Human Chemokine Kit - 552950	BD CBA Kit
<input type="checkbox"/> Human Inflammatory Cytokine Kit - 55...	BD CBA Kit
<input type="checkbox"/> Human Th1/Th2 Cytokine Kit - 550719	BD CBA Kit
<input type="checkbox"/> Human Th1/Th2 Cytokine Kit II - 551809	BD CBA Kit
<input type="checkbox"/> Human Th1/Th2/Th17 Cytokine Kit - 5...	BD CBA Kit
<input type="checkbox"/> Mouse Inflammation Kit - 552364	BD CBA Kit
<input type="checkbox"/> Mouse Th1/Th2/Th17 Cytokine Kit - 5...	BD CBA Kit
<input type="checkbox"/> Non-Human Primate Th1/Th2 Cytokine...	BD CBA Kit
<input type="checkbox"/> Mouse Immunoglobulin Isolating Kit - ...	BD CBA Kit

#Beads: 6

2. Select one or more beads from the Library list and do one of the following:
 - Drag and drop them onto the **Selected Beads** window.
 - Click **Add to Plex** in the **Bead and Model Selection** group on the ribbon.
 - If you have a bead group defined in the bead library, you can add all beads of the selected group in one step by adding the group to the plex. Select the group by clicking its name, then drag and drop onto the **Selected Beads** window.

Selecting the analysis model

There are three analysis models:

- Quantitative
- Qualitative
- Quality

Each bead has one analyte and optionally a second reporter parameter. The analysis model for an analyte is set to Quantitative by default.

To select the analysis model, do one of the following:

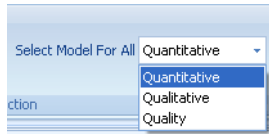
- Select the analysis model from the menu in the **Model** column.

This changes the analysis model for the selected bead only.

Analyte		
Name	Model	2nd Reporter
Human LT-alpha	Quantitative	No
Human Angiogenin	Quantitative	No
Human IL-8	Qualitative	No
Human IL-7	Quality	No
Human IL-6	Quantitative	No
Human IL-4	Quantitative	No

- Select the analysis model from the menu in the **Select Model For All** on the ribbon.

This changes the analysis model for all beads.



Next step

Proceed to [Selecting instrument settings \(page 53\)](#).

More information

- [Workflow overview \(page 24\)](#)
 - [Using the bead library \(page 104\)](#)
 - [Plex creation workflow \(page 50\)](#)
-

Selecting instrument settings

Introduction

This topic describes how to apply instrument settings. This process includes selecting a sample data file based on the defined plex, and specifying the scatter, clustering, and reporter parameters.

Requirements

To complete the instrument settings and cluster assignment process, you must have a FCS file that was acquired for the experiment and assigned to a sample in your plex.

About instrument settings

For common instruments, parameter options (scatter, clustering, reporter) are loaded into the menus automatically based on the selected file. Parameters can also be selected manually. When you have selected the parameters and change the FCS file, the scatter histogram and the clustering plot refresh according to the selected file.

Selecting instrument settings

To select instrument settings:

1. Click **Instrument Settings** in the **Plex** navigation panel.
2. Select any of the assigned files from the **Selected File** menu.

The scatter peak is set to one by default. You can change the value according to the number of different bead sizes used in your assay. The applied algorithm searches for the number of scatter peaks you defined. All BD CBA assays use one scatter peak.

If the algorithm fails to find the defined number of peaks, FCAP Array notifies you about the problem.

The clustering parameters are used to locate the beads, and the recommended clustering parameters can be found in the reagent manual.

The clustering algorithm finds the defined clusters. In case of failure, FCAP Array notifies you about the problem.

3. Select a parameter from the **Scatter Parameter** menu.

The scatter diagram shows the data.

4. Select a number from the **Scatter Peaks** menu.
5. Select the first and optionally the second clustering parameter from the **Clustering Parameters** menus according to the fluorescence of the beads.

For kits that use only one clustering parameter, you can clear the second clustering parameter selection by clicking the red **X** icon next to the menu.

6. Select a parameter from the **Reporter Parameter 1** menu for the bead analyte.
7. If your plex contains a second reporter parameter, select a parameter from the **Reporter Parameter 2** menu.

You can clear the second reporter parameter selection by clicking the red **X** icon next to the menu.

Next step

Proceed to [Assigning beads to clusters \(page 55\)](#).

More information

- [Plex creation workflow \(page 50\)](#)
 - [Defining controls \(page 72\)](#)
-

Assigning beads to clusters

Introduction

This topic describes how to assign the plex beads to clusters.

About assigning beads to clusters

You have to assign plex beads to the corresponding clusters in the clustering plot. To simplify assignment, first click the **Bead** column header in the **Select Beads** table to sort by bead ID.

Note that BD CBA kits are clustered left to right, in one dimension. Bead 1 on the left-most position is the dimmest bead.

You can assign only one bead to one cluster at a time.

Assigning beads to clusters

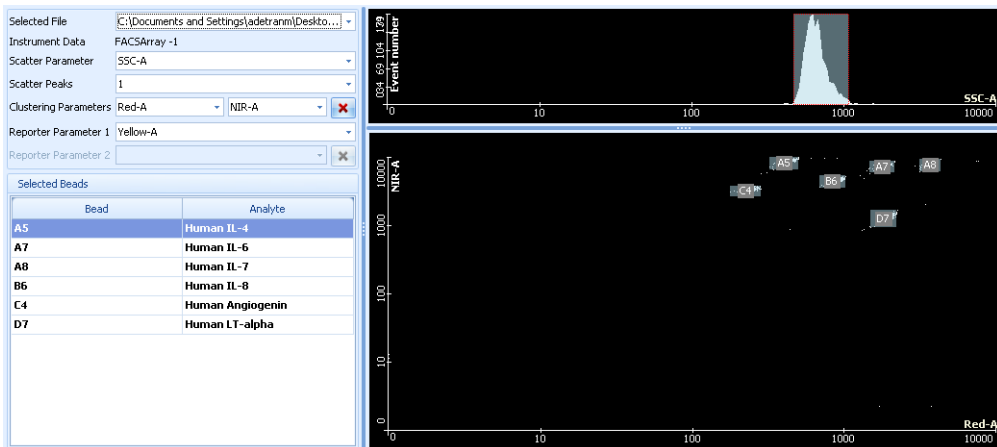
To assign beads to clusters:

1. Select the bead you want to assign from the **Selected Beads** table.

Sort by bead location and assign from the top left cluster. Assign beads going left to right and top to bottom.

2. Select the cluster by doing one of the following:
 - Double-click the cluster that you want to assign the bead to.
The label of the analyte is displayed on the cluster.
 - Drag and drop the selected bead onto a cluster.
3. Repeat this process for all of the clusters.

The following figure shows the clustering plot after beads have been assigned.



Removing bead assignments

To clear the bead cluster assignments, do one of the following:

- Click the **Clear Cluster Assignment** icon on the ribbon to clear all bead cluster assignments.
- To remove individual bead cluster assignments, right-click the individual cluster and select **Remove Bead Assignment**.

Next step

If the automatic clustering is satisfactory, then go to [Defining standards and QC \(page 64\)](#).

If you wish to adjust your gates, then go to one of the following:

- [Using debris filtering \(page 57\)](#)
- [Using manual clustering \(page 59\)](#)

More information

- [Plex creation workflow \(page 50\)](#)
- [Viewing standard curves \(page 74\)](#)

Using debris filtering

Introduction

This topic describes how to use debris filtering.

You can use this function to filter out debris that could affect the automatic clustering algorithm and lead to incorrect clustering results.

**Procedure**

To filter out debris from a plot, you draw a gate in the left plot (**Before Filtering** plot) around the area that you want to keep in the plot. The right plot (**After Filtering** plot) displays the results of the gate in the left plot.

The drawn gate(s) applies to all samples and is saved with the plex.

The **Apply Filter** icon in the **Debris Filtering** group toggles the filter on and off in the plot on the right side.

To use debris filtering:

1. Click **Debris Filtering** in the **Plex** navigation panel.

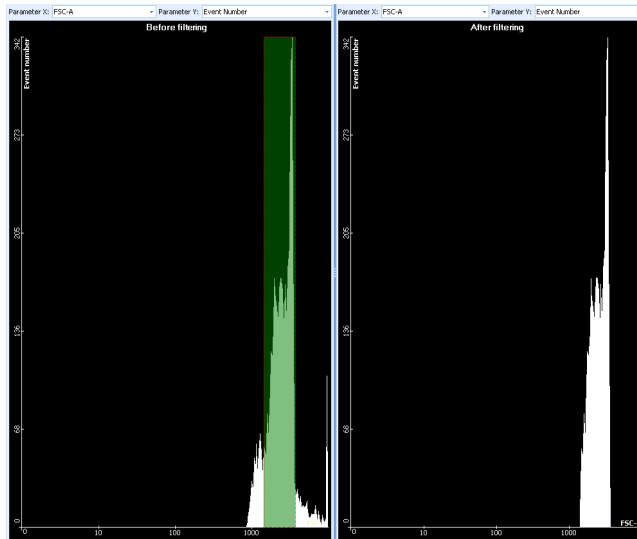
A list of samples and two plots are displayed.

2. Select a sample from the list.

The histograms are displayed according to the selected parameter of the selected sample file.

3. If needed, change the selected parameters by selecting from the menus above the histograms. You can use different parameters in the two plots.
4. On the left histogram, hold down the mouse button and move the mouse left or right to draw your gate. You can draw multiple gates. The histogram on the right shows the filter results.
5. (Optional) Gates can be moved, resized, and deleted.
 - Move a gate by selecting it and dragging it to another position.
 - Resize a gate by grabbing one of the sides.
 - Delete a gate by selecting it and clicking the **Remove Selected Gate** icon. You can also press the Delete key.

- The debris filter removes your assignment of beads to populations, so you need to re-assign them in the **Instrument Settings** or **Manual Clustering** views.



Next step

Proceed to [Assigning beads to clusters \(page 55\)](#).

More information

- [Plex creation workflow \(page 50\)](#)
 - [Using manual clustering \(page 59\)](#)
-

Using manual clustering

Introduction

This topic describes how to use manual clustering.

You can use this function to resolve problems caused when the automatic clustering process fails to locate your bead clusters. Automatic bead clusters can also be modified with this method.

The functions located in the **Manual Clustering** group can be used to navigate up or down the sample list and to perform actions on multiple samples at once.



Applying manual clustering to selected samples

To apply manual clustering to selected samples:

1. Click **Manual Clustering** in the **Plex** navigation panel.

The list of samples opens on the left, and two plots open on the right.

2. Review the **Clusters** column in the sample list table, where you can find out if manual clustering is needed for any of the samples.

The text turns red for any tube that fails auto clustering.

3. Select a sample from the list.

Data according to the scatter parameter selected in the **Instrument Settings** view is shown in the histogram. Data corresponding to the clustering parameters is shown in the dot plot. In the dot plot, you can check which cluster was not found by the algorithm.

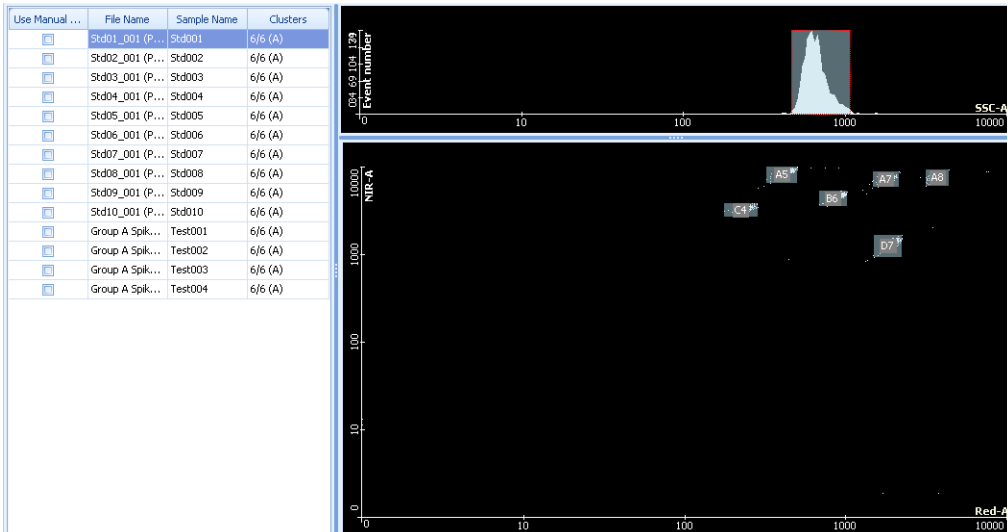
4. Change the auto cluster to manual cluster by selecting the **Use Manual Clustering** checkbox in the row of the selected sample.
5. On the histogram, hold down the mouse button and move the mouse left or right to draw your gate. You can draw multiple gates.
6. (Optional) Gates can be moved, resized, and deleted.
 - Move a gate by selecting it and dragging it to another position.
 - Resize a gate by grabbing one of the sides.
 - Delete a gate by selecting it and pressing the Delete key.

7. Use the mouse to draw a new gate around the cluster in the dot plot. Do one of the following:
 - Hold the mouse button while drawing your freehand gate.
 - Sequentially click to draw the sides of a polygon gate.
8. (Optional) To resize a dot plot gate, hold Ctrl + drag one of its sides.
9. After defining all the clusters, assign beads to them.
 - a. Select the bead you want to assign from the **Selected Beads** table.
 - b. Double-click the cluster that you want to assign the bead to.

Clearing manual clustering from selected samples

To change back to auto clustering for selected samples:

1. Clear the **Use Manual Clustering** checkboxes for your selected samples in the list.



Navigating the sample list

To navigate the sample list, do one of the following:

- Click a row in the sample list to show the file's sample data.
 - Click the **Next File** icon to move down the sample list and show the next file's sample data.
 - Click the **Previous File** icon to move up the sample list and show the previous file's sample data.
-

Selecting failed files for manual clustering

To select failed files for manual clustering:

1. Click the **Use Manual Clustering for Failed Files** icon.

You can now edit the failed files indicated by red colored text. Adjust scatter parameters, adjust clustering gates, and assign beads.

Selecting all files for manual clustering

To select all files for manual clustering:

1. Click the **Use Manual Clustering for All Files** icon.

You can now edit all files. Adjust scatter parameters, adjust clustering gates, and assign beads.

Reverting to auto clustering for all files

To revert back to automatic clustering for all files:

1. Click the **Use Auto Clustering for All Files** icon.

All parameters are restored to automatic clustering.

Applying manual clustering to selected files

To apply manual clustering to selected files:

1. Select one or more files from the sample list by clicking the corresponding checkboxes.
2. Draw a clustering gate(s), adjust scatter parameter(s), or assign beads.
3. Click the **Apply to All Manual Clustering Files** icon.

All your parameter changes are applied to all selected files.

Clearing the bead assignments

To clear the bead assignments:

1. Select a file from the sample list by clicking the corresponding checkbox.
2. Click the **Clear Cluster Assignment** icon.

All the bead assignments for the selected file are removed. The scatter parameter and clustering gates remain unchanged.

Note that if you select multiple checkboxes in the sample list, only the sample in the highlighted row is affected when you click the **Clear Cluster Assignment** icon.

Clearing all bead assignments and all clustering gates

To clear all bead assignments and all clustering gates:

1. Select a file from the sample list by clicking the corresponding checkbox.
2. Click the **Clear All Bead Clusters** icon.

All bead assignments and all clustering gates are removed. The scatter parameter remains unchanged.

Note that if you select multiple checkboxes in the sample list, only the sample in the highlighted row is affected when you click the **Clear All Bead Clusters** icon.

Next step

Proceed to [Defining standards and QC \(page 64\)](#).

More information

- [Plex creation workflow \(page 50\)](#)
 - [Using debris filtering \(page 57\)](#)
-

Defining standards and QC

Introduction

This topic describes how to define standards for quantitative, qualitative, and quality analysis. It also describes how to use the sample based QC method for the BD CBA Mouse Immunoglobulin Isotyping kit.

Viewing standards

To view standards:

1. Click **Standards and QC** in the **Plex** navigation panel.

The content appears corresponding to the selected beads and their analytes' analysis models.

When your plex contains a second reporter parameter, the window is divided into two panels.

About quantitative analysis

When defining standards for quantitative analysis, you specify these items:

- Concentration values
- Measurement unit

Specifying the standard concentration

You can use uniform concentrations for all analytes (default) or specify different concentrations for each analyte.

To specify the standard concentration:

1. Click **Ascending** or **Descending** in the **Concentration Settings** group to define the order for standard concentrations.

The typical choice here is ascending, since most reagent instructions recommend running from lowest to highest concentration.



2. Enter a value in the **Highest Concentration** field.

3. Enter a value in the **Dilution Factor** field.
4. Click **Use Blank** if you need a zero concentration point.
5. Click **Apply**.

If the icon is not available, click the column header.

The program calculates the standard dilution series and fills in the concentration values. You can also type in the values manually.

Reporter Parameter 1	
Standard Samples of Quantitative Analysis	
Standard Sample	Concentration
Std001	0.00 pg/mL
Std002	19.53 pg/mL
Std003	39.06 pg/mL
Std004	78.13 pg/mL
Std005	156.25 pg/mL
Std006	312.50 pg/mL
Std007	625.00 pg/mL
Std008	1,250.00 pg/mL
Std009	2,500.00 pg/mL
Std010	5,000.00 pg/mL

Show Individual Analytes

Adding concentration settings for an individual analyte

You can modify individual concentrations manually by editing or using the following procedure.

To add concentration settings for an individual analyte:

1. Select the **Show Individual Analytes** checkbox.
2. Click in the column header for an individual analyte.
3. Enter a value in the **Highest Concentration** field.
4. Enter a value in the **Dilution Factor** field.
5. Click **Use Blank** if you need a zero concentration point.
6. Click **Apply**.

Only the selected analyte's concentration column is calculated.

- Repeat this process to define the concentrations for each analyte.

Reporter Parameter 1

Standard Samples of Quantitative Analysis

Sample Name	D7-Human LT-alpha	C4-Human Angiogenin	B6-Human IL-8	A8-Human IL-7	A7-Human IL-6	A5-Human IL-4	
Std001	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	
Std002	19.53 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	
Std003	39.06 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	
Std004	78.13 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	
Std005	156.25 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	
Std006	312.50 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	
Std007	625.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	
Std008	1,250.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	
Std009	2,500.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	
Std010	5,000.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	0.00 pg/mL	

Show Individual Analytes

Specifying the measurement unit

When defining concentrations, the active measurement unit will be valid for your analysis.

To change the measurement unit:

1. Select a **Measurement Unit** in the **Concentration Settings** group.
2. Click the **Concentration** column header to highlight it.
3. Click **Apply**.

The selected measurement unit is displayed in the results and report documents.

Note that using different measurement units gives slightly different results for your standard curves. We recommend not using units that will lead to a concentration of <1 .

About qualitative analysis

When using qualitative analysis, you can select positive and negative standards. You can use positive and negative standards for all analytes (default) or specify standards for each analyte. All analytes should have a positive or negative standard.

Specifying positive and negative standards

To specify positive and negative standards:

1. Select negative standards in the negative row of the qualitative table by selecting a standard sample name from the **Standard Sample** menu.

Qualitative Type	Standard Sample
Negative	Std001
Positive	Std002
	Std001
	Std002
	Std003
	Std004
	Std005
	Std006
	Std007

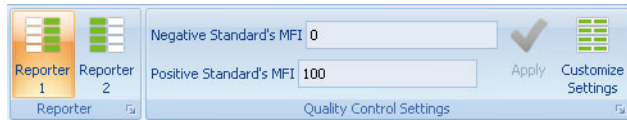
2. Use the same method for positive standards.

About quality control analysis

With quality control analysis, you have the option to check if MFI values are within the specified range. Values that are out of range are indicated in the results and reports.

The quality control range is uniform for all analytes by default.

In addition to using the following procedures, you can manually edit each value.



Specifying MFI values for quality control

To specify quality control MFI threshold values for all analytes:

1. Click a column header.
2. Type values into the **Negative Standard's MFI** and **Positive Standard's MFI** fields.
3. Click **Apply**.

The columns are filled with the minimum and maximum values for all samples.

- If you have a second reporter parameter, click the **Reporter 2** icon and repeat steps 1 to 3.

Reporter Parameter 1

Quality Control Definition

Sample Name	Neg MFI Value	Pos MFI Value
Std001	0.00	100.00
Std002	0.00	100.00
Std003	0.00	100.00
Std004	0.00	100.00
Std005	0.00	100.00
Std006	0.00	100.00
Std007	0.00	100.00
Std008	0.00	100.00
Std009	0.00	100.00
Std010	0.00	100.00

Show Individual Analytes

Specifying MFI values for an individual analyte

To specify quality control MFI threshold values for individual analytes:

- Select the **Show Individual Analytes** checkbox.
- Click the column header for an individual analyte.
- Type values into the **Negative Standard's MFI** and **Positive Standard's MFI** fields.
- Click **Apply**.
- Repeat this process to specify the MFI threshold values for each analyte.
- If you have a second reporter parameter, click the **Reporter 2** icon and repeat steps 1 to 5.

About sample based QC

Using the sample based QC definition enables the minimum MFI values to be derived from the MFI values of the negative standard.

You can define your standard as negative, positive, or both a negative and a positive standard by clicking the corresponding tab in the Sample Based QC Definition dialog.

The results for your analysis can be found in the QC Result column of the statistics table in the report view.

Defining sample based QC for all samples

Use the following method for the analysis of the BD CBA Mouse Immunoglobulin Isotyping kit.

To define sample based QC Definition:

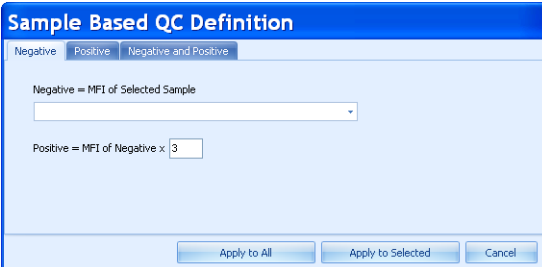
1. Click the **Customize Settings** icon in the **Quality Control Settings** group.

The **Sample Based QC Definition** dialog opens.

2. Select your negative standard from the **Negative=MFI of Selected Sample** menu.
3. Specify the multiplier for your negative standard in the **Positive=MFI of Negative x** field.

We recommend a value of 3 for the multiplier.

4. Click **Apply to All**.



The screenshot shows the "Sample Based QC Definition" dialog box. It has three tabs: "Negative", "Positive", and "Negative and Positive". The "Negative" tab is selected. The dialog contains a dropdown menu labeled "Negative = MFI of Selected Sample" and a text input field labeled "Positive = MFI of Negative x" with the value "3" entered. At the bottom, there are three buttons: "Apply to All", "Apply to Selected", and "Cancel".

- Click the **Reporter 2** icon and repeat steps 1 to 4.

Verify that you have the correct **Reporter** icon highlighted on the ribbon while performing this procedure. Toggling between the **Reporter** icons will toggle between the two **Quality Control Definition** tables.

The software provides results for each analyte and reporter.

Result	Definition
Positive	The MFI of the sample is greater than the (user-defined multiplier) times the MFI of the negative standard.
Negative	The MFI of the sample is less than or equal to the MFI of the negative standard.
Indeterminate	The MFI of the sample is greater than the MFI of the negative standard, but less than the (user-defined multiplier) times the negative standard.
Error	Calculation error.

Defining sample based QC for an individual analyte

Verify that you have the correct file(s) selected when performing this procedure. Calculations are based on the selected negative standard.

To define sample based QC for an individual analyte:

- Select the **Show Individual Analytes** checkbox.
- Click a column header of an individual analyte.
- Click the **Customize Settings** icon in the **Quality Control Settings** group.

The **Sample Based QC Definition** dialog opens.

- Select your negative standard from the **Negative=MFI of Selected Sample** menu.
- Specify the multiplier for your negative standard in the **Positive=MFI of Negative x** field.

6. Click **Apply to Selected**.

Next step

Proceed to [Defining controls \(page 72\)](#).

More information

- [Plex creation workflow \(page 50\)](#)
 - [Selecting beads and analysis model \(page 51\)](#)
-

Defining controls

Introduction

This topic describes how to add control samples to a plex for quantitative and qualitative analysis.

About defining controls

You can specify the parameters of the control samples and then use them in a plex to verify the accuracy of the measurement and the calculations.

Controls added to the plex are displayed in the quantitative or qualitative analysis table, corresponding to the selected analyte model.

Defining controls in a quantitative analysis

To define controls in a quantitative analysis:

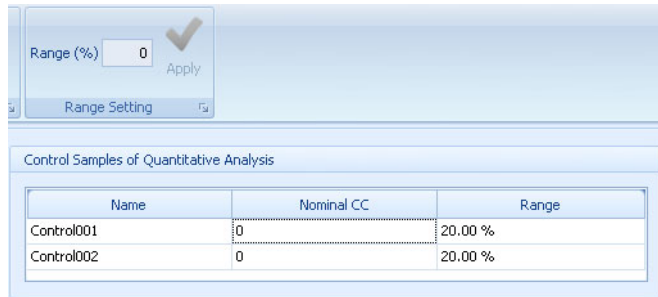
1. Click **Control Definition** in the **Plex** navigation panel.
2. Specify the concentration of your control samples by clicking in the **Nominal CC** field and entering the value.

The measurement unit matches the one defined in **Standards and QC**.

3. If needed, change the **Acceptance Range** value.

It is set to 20% by default (Final CC +/- 20%). Enter a value between zero and one to change it.

Values that are out of range are indicated in the results and reports.

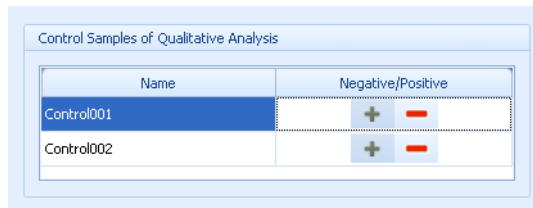


Defining controls in a qualitative analysis

To define controls in a qualitative analysis:

1. Click **Control Definition** in the **Plex** navigation panel.
2. Define the control samples as negative or positive by clicking the appropriate sign in the **Negative/Positive** column.

Values that are out of range are indicated in the report and the results.



Next step

Proceed to [Viewing standard curves \(page 74\)](#).

More information

- [Plex creation workflow \(page 50\)](#)
- [Defining standards and QC \(page 64\)](#)

Viewing standard curves

Introduction

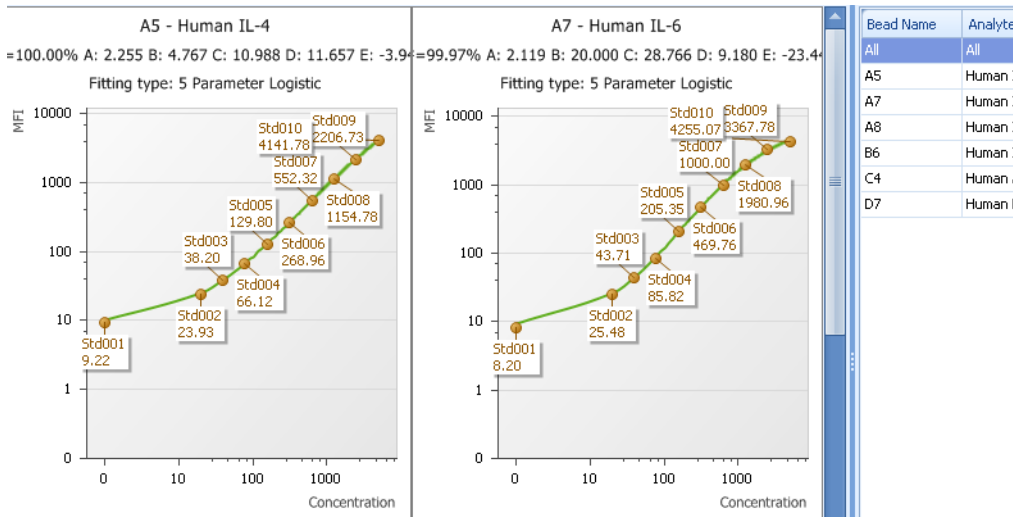
This topic describes how to view the standard curves and how to modify the display to optimize the views for an experiment.

Viewing the standard curves

To view the standard curves:

1. Click **Standard Curves** in the **Plex** navigation panel.

The window is separated into two panels: curves on the left and the analytes table on the right. The following figure shows a portion of a set of standard curves.



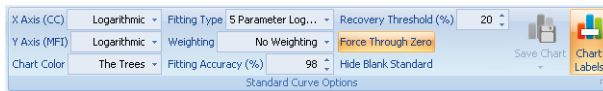
2. If needed, zoom in on the standard curve and statistics for a specific analyte either by selecting an analyte from the **Analytes** list or by double-clicking a plot.

The fitted results statistics are displayed below the plot.

Note that in an experiment with multiple replicates of a standard sample, the results table shows the average values first, and the values for the replicates in the rows below.

Standard curve options

You can modify the parameters of the calculated standard curves by using the features displayed in the **Standard Curves Options** group.



Changing the axis scaling

The scales of the x-axis and y-axis are set to **Logarithmic** by default.

To change axis scaling:

1. Click **Linear** in the **X Axis** or **Y Axis** menu.

Changing the fitting type

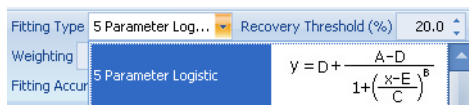
The fitting type is **5 Parameter Logistic** by default.

To change the fitting type:

1. Select another type from the **Fitting Type** menu.

The recalculation of the curves runs automatically, and the plots refresh to show the new data.

You can find the equation of each fitting type in the list.



Changing the weighting type

FCAP Array calculates standard curves without weighting by default.

There are two weighting models available: based on event numbers and on event numbers/CV values.

To change weighting:

1. Select a weighting model from the **Weighting** menu.

After you select one of the models, the recalculation runs automatically and the plots refresh the data.

Changing the fitting accuracy percentage

If the R square of the fitting algorithm result is lower than the value of the fitting accuracy, you receive an error message regarding the related analyte. Fitting accuracy is set to 98% by default.

To change the fitting accuracy percentage:

1. Change the **Fitting Accuracy (%)** value in the **Standard Curve Options** group either by entering a new number or using the spin button and then pressing Enter.
-

Changing the force through zero option

If you have a blank standard sample (0 pg/mL) in the plex, you have an option to ignore the data in the fitting calculation. The **Force Zero** option in the **Standard Curve Options** group is enabled by default.

To change the force through zero option:

1. Click the **Force Zero** button on the **Standard Curve Options** group.
-

Hiding the blank standard**To hide the blank standard on the standard curves:**

1. Click the **Hide Blank Standard** icon.

The blank standard is hidden on all curves, but the corresponding file is still present in the analysis.

Changing the recovery threshold percentage

To change the recovery threshold percentage:

1. Change the **Recovery Threshold (%)** value in the **Standard Curve Options** group either by entering a new number or using the spin button and then pressing Enter.
-

Saving the chart

When displaying a single curve, the **Save Chart** icon is active.

You can adjust the image size to change the proportions of the saved chart. The saved chart image will appear the same way it does on the screen.

To save the chart:

1. Click the **Save Chart** icon to save the active chart to an image file.
2. Select a file format from the menu.

The choices are JPEG, PNG, and BMP.

The **Save As** dialog opens.

3. Designate a file location and name.
 4. Click **Save**.
-

Changing the chart color

To change the chart color:

1. Select a chart color from the menu on the **Standard Curve Options** group.

When saving a curve, the image file corresponds to the selected color.

Changing the chart labels option

To change the chart labels option:

1. Click the **Chart Labels** icon on the **Standard Curve Options** group to toggle the display of standard point descriptors (standard point name and respective MFI value) on the chart.

Ignoring standard points

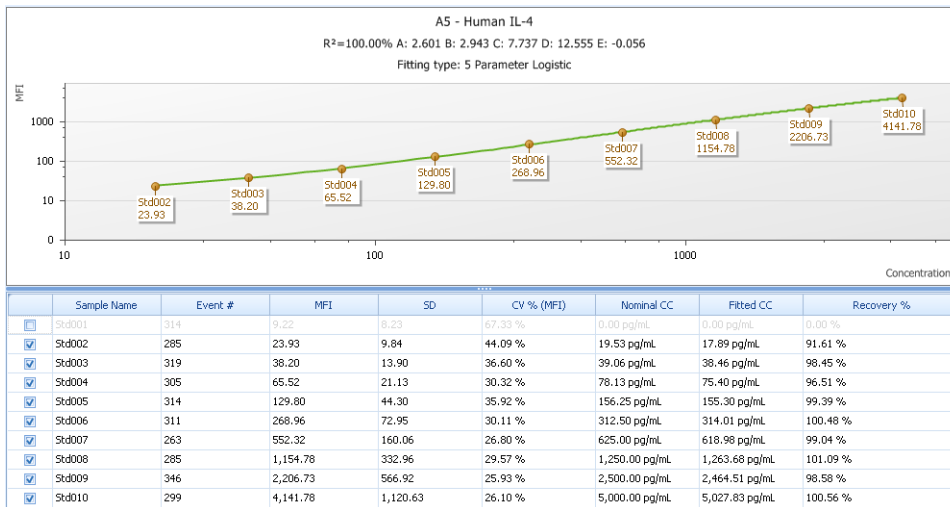
You can view the standard curves and ignore one or more standard points in case they do not correctly fit the curves.

Clearing the checkbox of a standard point ignores the standard point from all curves, not just the one being viewed.

To ignore standard points on the standard curve:

1. Go to the detailed view.
2. Clear the checkbox in the results table of the standard point you want to ignore.

The recalculation runs automatically and the results and the plots reload with the new data.



Next step

- Proceed to [Overview of results and reports \(page 80\)](#).

More information

- [Plex creation workflow \(page 50\)](#)

6

Results and reports management

This chapter covers the following topics:

- [Overview of results and reports \(page 80\)](#)
- [Viewing analysis results \(page 81\)](#)
- [Customizing charts \(page 87\)](#)
- [Viewing reports \(page 89\)](#)
- [Completing an experiment \(page 91\)](#)

Overview of results and reports

Introduction

This topic describes the stages in the workflow for viewing analysis results and creating reports.

8	Results per Analyte
9	Results per Sample
10	Report

Results and reports workflow

The workflow for viewing results, creating reports, and completing an experiment includes these stages.

Stage	Description
1	Viewing analysis results (page 81)
2	Customizing charts (page 87)
3	Viewing reports (page 89)
4	Completing an experiment (page 91)

More information

- [New experiment overview \(page 28\)](#)
 - [Workflow overview \(page 24\)](#)
-

Viewing analysis results

Introduction

This topic describes how to review the results of an analysis by inspecting the analysis statistics and histograms.

Two types of results views are available:

- Results per analyte
 - Results per sample
-

Viewing results

To view results:

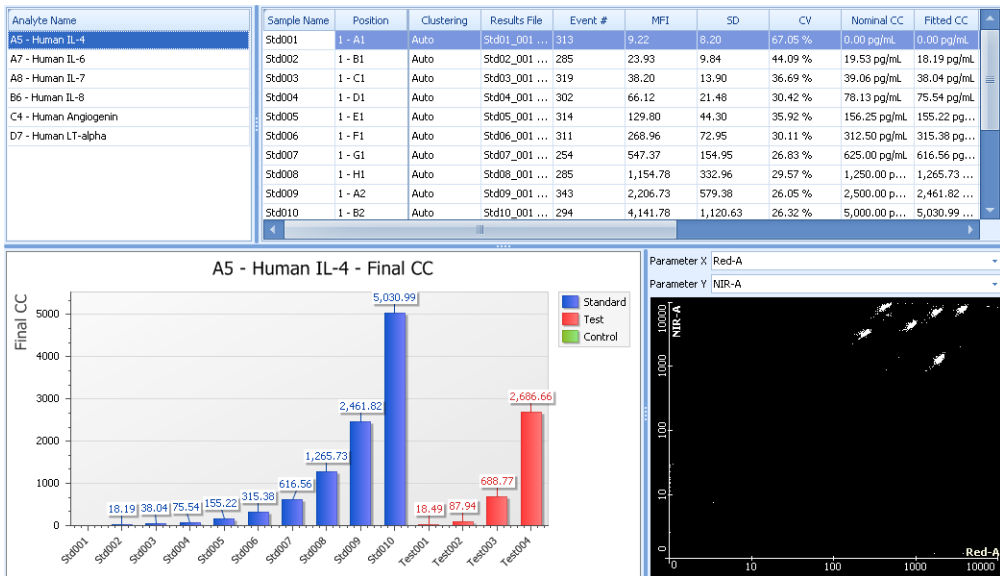
1. Click one of the views in the **Plex** navigation panel.

The results data is displayed in two tables at the top and in charts and plots below.

For replicate samples, the black lines that are shown on the bar chart depict a range bar. It extends from the highest point to the lowest point.

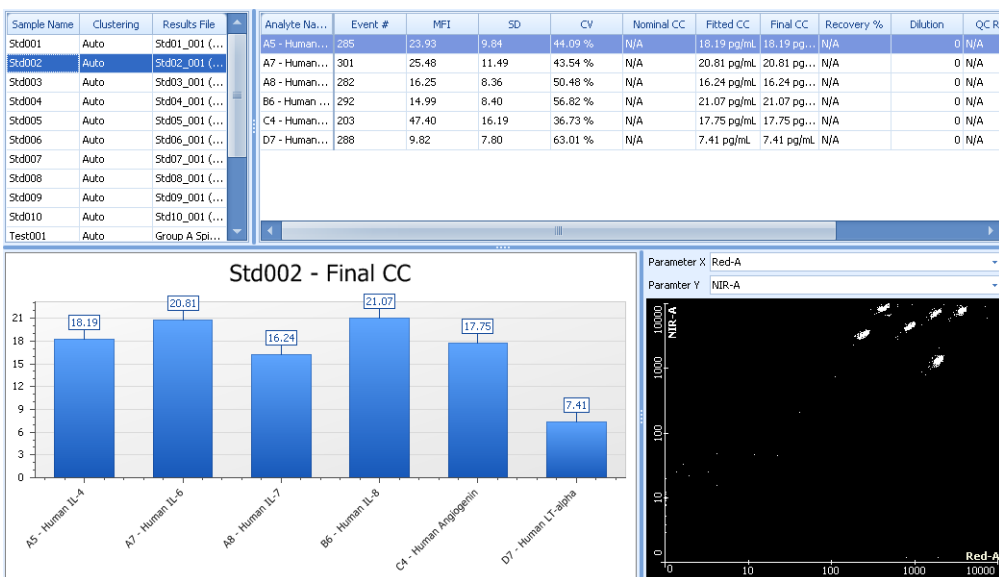
Results per analyte This view shows the list of analytes in the left table and the statistics for the selected analyte in the right table. Use the horizontal scroll bar in the right table to see all of the statistics columns.

In the following figure, the plot shows results for the analyte selected from the left table.



Results per sample This view shows the list of samples in the left table and the statistics for the selected sample in the right table. Use the horizontal scroll bar in the right table to see all of the statistics columns.

In the following figure, the plot shows results for the analyte selected from the left table.



Column headers

The statistics table has columns for each of the categories of data. The following table describes the meaning for each column.

Column	Description
Position	The location of the sample on the plate in the experiment design view
Results file	Assigned FCS data file
Clustering	Type of clustering used (manual or auto)
Event #	The number of events in the gated cluster
MFI	The median fluorescence intensity
SD	The standard deviation of the reporter MFI for the bead cluster
CV	Coefficient of variation as a percentage of the reporter MFI of a cluster
Nominal CC	Nominal concentration of the standard or control samples
Fitted CC	Sample concentration calculated from the fitting curves
Final CC	Sample concentration calculated from the fitting curve with a dilution factor applied
Recovery %	Applicable to standard sample only. It represents the comparison of the nominal CC to the fitted or final CC
Dilution	Dilution factor
QC	Quality Control analysis result message
Qualitative	Qualitative analysis result message
Message	Shows error message for the sample in the corresponding row

Organizing the data in the statistics table

You can organize the order of the data in the columns in the statistics table to decreasing or increasing.

To organize data:

1. Click the column header.
2. Click the arrow in the header to change the order of the data.

Setting up custom filtering in the statistics table

You can set the criteria for data that is displayed and hide rows that you do not want to show by using the custom filter. You can then export this filtered data.

To set up custom filtering:

1. Click the filter icon in the right corner of the column header to open the menu.

Analyte Name	Event #	MET
A5 - Human IL-4	285	(Custom)
A7 - Human IL-6	301	(Blanks)
A8 - Human IL-7	282	(Non blanks)
B6 - Human IL-8	292	203
C4 - Human Angiogenin	203	282
D7 - Human LT-alpha	288	285
		288
		292
		301

2. Select **Custom**.

The Custom Auto Filter dialog opens.

3. Complete the fields to filter the data.

4. Click **OK**.

The filter is displayed at the bottom of the table where you can modify, inactivate, or delete the filter.

Exporting results

The data from results tables can be exported as different file formats, including Microsoft Excel® (XLS), PDF, RTF, HTML, and CSV. The file format is set to XLS by default. Click the arrow on the **Export** icon to change it.

To export results:

1. Click the **Export** icon in the **Results** group.
The **Save As** dialog opens.
 2. Select a destination to save the file.
 3. Click **Save**.
-

Printing

To print the results tables:

1. Click the **Print** icon in the **Results** group.
 2. If necessary, edit the printer and page setup in the **Print Preview** dialog.
 3. Click the **Print** icon.
-

Next step

Proceed to [Customizing charts \(page 87\)](#).

More information

- [Overview of results and reports \(page 80\)](#)
 - [Workflow overview \(page 24\)](#)
-

Customizing charts

Introduction

This topic describes how to customize the display of results data in the graphical charts.

You can select a row in the left table to display the data in the chart. When the view is set to **Results per Analyte**, the chart displays the data for each sample for a given analyte. When the view is set to **Results per Sample**, the chart displays the data for each analyte for a given sample.

Displaying different sample types in the results per analyte view

To change the sample type view on the chart:

1. Click the corresponding icons (**Standard**, **Test**, or **Control**) in the **Results** group.

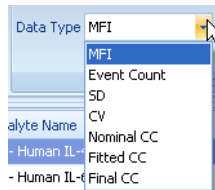
Displaying the different data types

The data type displayed in the chart is set to **Final CC** by default.

To change the data type:

1. Select a data type from the **Data Type** menu in the **Chart** group.

The menu contains all the listed data types from the results table.



Changing the chart type

To change the chart type:

1. Click the **Chart Type** icon in the **Chart** group.
2. Select one of the following: **Bar Chart**, **3D Bar Chart**, or **Polar Chart**.

When you select **3D Bar Chart**, after clicking the chart, you can rotate it with your mouse and zoom in or out with your mouse wheel.

Changing the chart labels option

To change the chart labels option:

1. Click the **Chart Labels** icon on the **Chart** group to toggle the display of descriptors on the chart.

Saving a chart

To save a chart:

1. Click the **Save Chart** icon in the **Chart** group.

The **Save As** dialog opens.

You can adjust the image size to change the proportions of the saved chart. The saved chart image will appear the same way it does on the screen.

The format is set to JPEG by default. BMP and PNG formats are available as well.

2. Select a destination to save the file.
3. Click **Save**.

Next step

Proceed to [Viewing reports \(page 89\)](#).

More information

- [Overview of results and reports \(page 80\)](#)
 - [Workflow overview \(page 24\)](#)
-

Viewing reports

Introduction

This topic describes how to work with reports.

Two types of report documents are available in FCAP Array: plex reports and experiment reports. You can view and print both types. Report documents contain all the defined data for analysis and results.

The plex report contains all the details of the selected plex such as sample layout, beads and model selection, standard and quality control definition, control definition, instrument settings, standard curves, and results.

The experiment report contains all the plex reports merged into a single document.

Viewing plex reports

To view a plex report:

1. Click **Report** in the **Plex** navigation panel.
-

Viewing experiment reports

To view an experiment report:

1. Click **Report** in the **Experiment** navigation panel.
-

Report layout and format

The options for working with reports are shown on the ribbon. The groups include:

- Print
- Page Setup
- Navigation
- Zoom
- Page background
- Export

You can modify the layout and the format of the report by clicking one of the icons. To see a description of the icon, hover the pointer over it to see a tooltip dialog.

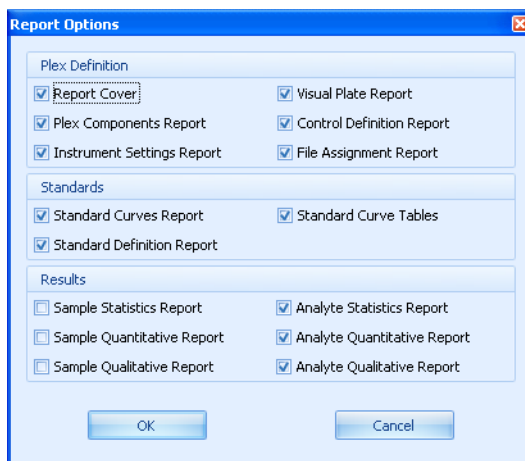
Using report content options

You can show or hide specific parts of a report.

To toggle report content options:

1. Click the **Options** icon in the **Page Setup** group.

The **Report Options** dialog opens.



2. Select or clear the options as necessary.

Any options that are selected are included in the report. You can turn options on or off at any time.

Note that the selected options will be applied to the current and future reports.

3. Click **OK**.

Next step

Proceed to [Completing an experiment \(page 91\)](#).

More information

- [Overview of results and reports \(page 80\)](#)
- [Workflow overview \(page 24\)](#)

Completing an experiment

Introduction

This topic describes the process of completing an experiment.

You can finish your work during any phase of the analysis. There are several options for completing an experiment. These functions are available from any window or view of the experiment.

If you are working with a locked experiment, saving is not active and modifications are also disabled.

Saving an experiment

To save an experiment:

1. Click the **Save** icon in the **Experiment** group on the ribbon.

FCAP Array saves all changes to the experiment.

Saving an experiment as

When working with a locked experiment, use this option to save a copy of it. The new copy of the locked experiment is now editable and your modifications can be saved. Using the **Save As** function, FCAP Array keeps the original experiment unmodified and saves your copy with the completed changes as well.

To use save experiment as:

1. Click the **Save As** icon in the **Experiment** group.

The **Save As** dialog opens.

2. Enter a name for the experiment.
3. Click **Save**.

Saving a plex as a template

You can reuse the saved template for your next experiment and streamline your workflow.

To save a plex as a template:

1. Click the **Save Plex as Template** icon in the **Experiment** group.
The **Save As** dialog opens.
2. Enter a name for the plex.
3. Click **Save**.

Closing an experiment

To close an experiment:

1. Click the **Close** icon in the **Experiment** group.

The experiment is closed automatically. The program returns to the main window showing the experiment library.

More information

- [Overview of results and reports \(page 80\)](#)
 - [Workflow overview \(page 24\)](#)
-

7

Using the BD FACSuite workflow

This chapter covers the following topics:

- [BD FACSuite workflow overview \(page 94\)](#)
- [Creating a new experiment from BD FACSuite files \(page 96\)](#)
- [Evaluating the experiment \(page 98\)](#)

BD FACSuite workflow overview

Introduction This topic describes the workflow for creating an experiment from BD FACSuite FCS files.

Workflow stages The following table shows the stages in a typical workflow when using FCS files from BD FACSuite software.

Stage	Description
1	Starting the software (page 17)
2	Creating a new experiment from BD FACSuite files (page 96)
3	Evaluating the experiment (page 98)
4	Viewing analysis results (page 81)
5	Viewing reports (page 89)

About CBA-specific keywords

To use the BD FACSuite workflow most efficiently, it is important that CBA-specific keywords be added to the experiment while it is being run in BD FACSuite software. See the *Guide to Using BD FACSuite Software with BD Cytometric Bead Array Products* to learn how to perform this setup.

Go to bdbiosciences.com/cbasetup for more BD CBA information.

Keyword	Description
CBA Plex Name	Identifies the specific plex from the FCAP Array software plex library. If a match is found in the plex library, the FCS files are automatically associated with that plex.
CBA Type	Identifies whether the tube or well contains a standard, sample, or control. FCAP Array software lists analysis results in the following order: standards, samples, controls.
CBA Standard ID	<ul style="list-style-type: none"> • ID of the standard contained in the tube or well. The value can also be Pos or Neg to identify positive and negative for qualitative BD CBA assays. • Standards will be arranged for the standard curve in alphabetical order by the value of the CBA Standard ID keyword. Assign this keyword appropriately to ensure that standards are plotted in the correct order on the standard curve. Use leading zeros in the CBA Standard ID and CBA Control ID keywords. This ensures that your files can always be sorted in order (01, 02, 03 as opposed to 1, 2, 3).
CBA Control ID	<ul style="list-style-type: none"> • ID of the control contained in the tube or well. The value can also be Pos or Neg. • Controls will be arranged in alphabetical order by the value of the CBA Control ID keyword.
CBA Dilution	<ul style="list-style-type: none"> • Used to specify the dilution of the sample. The default dilution value is 1.00. • Note that the dilution factor can be entered as a keyword or it can also be entered into FCAP Array software.

More information

- [Workflow overview \(page 24\)](#)

Creating a new experiment from BD FACSuite files

Introduction

This topic describes how to create a new experiment using FCS files created in BD FACSuite software.

Note that you cannot create a plex using the standard workflow and then try to use that plex in the BD FACSuite workflow. The keywords in the BD FACSuite FCS files are used to generate the plex(es) for the BD FACSuite workflow.

Procedure

To create a new experiment from FCS files created in BD FACSuite software:

1. Click the **New Experiment** button in the **Home** tab and select **New Experiment > From FACSuite Files**.



The **New Experiment from FACSuite files** dialog opens.

2. Navigate to the directory containing the appropriate FCS files.

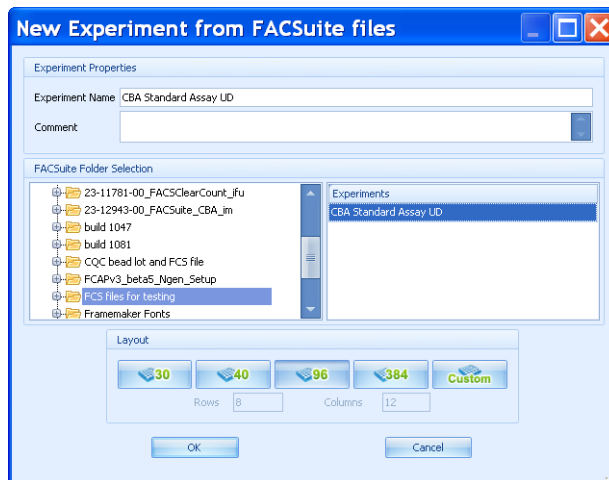
The FACSuite Experiment folder defined in **Preferences** is selected as the default in the **FACSuite Experiment Selection** browser.

The list of experiments in the right panel is the list of all unique \$PROJ keyword values extracted from all FCS files contained in the folder. The new experiment will be populated with all the FCS files that share the selected \$PROJ value.

3. Select the experiment name in the **Experiments** field to use the same name for the FCAP Array experiment.

A custom name can also be entered into the **Experiment Name** field.

You can also enter a comment.



4. Click **OK**.

The new experiment opens with the layout as defined by the keywords in the BD FACSuite FCS files.

Next step

Proceed to [Evaluating the experiment \(page 98\)](#).

More information

- [BD FACSuite workflow overview \(page 94\)](#)
 - [Workflow overview \(page 24\)](#)
-

Evaluating the experiment

Introduction	This topic describes the process of evaluating the experiment that is created after opening the BD FACSuite FCS files.
About generating experiment results	FCAP Array automatically generates plexes, samples, plates, and their layout, based on the information contained in the keywords in the BD FACSuite FCS files. FCAP Array creates a number of plexes to match those defined in the keywords. If the automatic analysis runs successfully based on the information, the experiment results are generated without defining any additional settings.
Verifying and adding information	<p>To verify or add information:</p> <ol style="list-style-type: none">1. Check the positions and types of samples in the experiment design view by clicking a well to see if it matches your corresponding BD FACSuite FCS file (Sample Properties panel). <p>While evaluating the created experiment, FCAP Array provides the option to select and add more samples, files, or plexes to it. You can also modify the properties of the previously added files. For further information, see Working with plexes (page 47).</p>
Solving problems	For cases where there is missing or an undefined plex name, you must define additional information. To learn more about how to solve this issue, see Working with plexes (page 47) or Troubleshooting (page 115) .
More information	<ul style="list-style-type: none">• BD FACSuite workflow overview (page 94)• Workflow overview (page 24)

8

Data management

This chapter covers the following topics:

- [Working with libraries \(page 100\)](#)
- [Using the experiment library \(page 102\)](#)
- [Using the bead library \(page 104\)](#)
- [Using the plex template library \(page 108\)](#)
- [Using the user management library \(page 111\)](#)
- [Using the units library \(page 113\)](#)

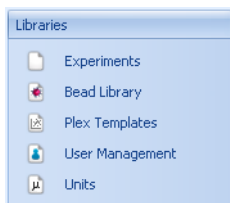
Working with libraries

Introduction

This topic describes how to work with the libraries.

About libraries

The available data from different parts of the application are organized in libraries. The default library in the main window is the experiment library. Use the **Libraries** panel to view the different libraries.



In each library, the data is displayed in tables, and the corresponding management icons display on the ribbon. Some of these functions are also available by right-clicking a table.

Editing data

To edit data:

1. Click a cell in the library table.
 2. Edit the data.
 3. Press Enter.
-

Sorting data

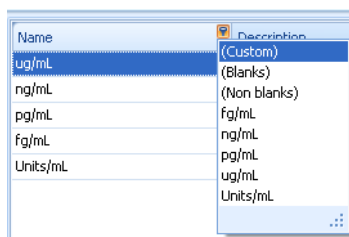
To sort data:

1. Click the column header.
2. Click the arrow in the header to change the order of the data.

Filtering data

To filter data:

1. Click the filter icon in the right corner of the column header to open the menu.



2. Do one of the following:
 - Select one of the options from the menu.
 - Specify your own parameters by clicking **Custom**, completing the fields, and clicking OK.

A screenshot of the 'Custom AutoFilter' dialog box. The title bar is blue with the text 'Custom AutoFilter' and a red close button. The main area is light blue and contains the following elements: 'Show rows where:' followed by 'Analyte Name'. Below this is a dropdown menu with 'like' selected and an input field with '(Enter a value)'. There are two radio buttons: 'And' (selected) and 'Or'. Below these is another dropdown menu with '(Select an operator)' selected and another input field with '(Enter a value)'. At the bottom left is an information icon (i), and at the bottom right are 'OK' and 'Cancel' buttons.

The filter is displayed at the bottom of the table, where you can modify, inactivate, or delete the filter.

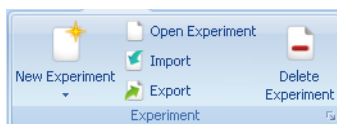
Using the experiment library

Introduction

This topic describes how to work with experiment files in the experiment library.

About experiment functions

The available experiments are organized in the experiment library. The functions are located in the **Experiment** group on the ribbon.



Creating an experiment

To create an experiment:

1. Click the lower half of the **New Experiment** icon.
2. Select an experiment type.

A dialog opens.

3. Enter any experiment details and click **OK**.

For more detailed instructions, see [Creating a new experiment \(page 29\)](#).

Opening an experiment

To open an experiment:

1. Select an experiment from the experiment table.

Name	Created By	Creation Date	Comment	Last Save D...	Last Save By	Locked
Experiment	Administrator	3/7/2011		3/7/2011	Administrator	No
cba	Administrator	3/7/2011		3/7/2011	Administrator	No

2. Click the **Open Experiment** icon.

Alternatively, you can double-click the selected experiment to open it.

Viewing experiment properties

To view experiment properties:

1. Select an experiment from the experiment table.

The properties table displays the details for the selected experiment.

Properties	
Name	Project 1
Created By	Administrator
Creation Date	4/29/2011 11:13 AM
Last Save By	Administrator
Last Save Date	4/29/2011 11:13 AM
Comment	
Conditions	
Institute	
Responsible	
Operator	
Access State	OwnersOnly
Number of Plates	1
Last Print Date	

Deleting an experiment

To delete an experiment:

1. Select an experiment from the experiment table.
2. Click the **Delete Experiment** icon.

The **Confirm experiment deletion** dialog opens.

3. Click **Yes**.

Importing an experiment

Experiments can be shared between different computers using the experiment import and export functions.

To import an experiment:

1. Click the **Import** icon.

The **Import Experiment** dialog opens.

2. Navigate to your experiment file and click **Open**.

Exporting an experiment**To export an experiment:**

1. Select an experiment from the experiment table.
2. Click the **Export** icon.

The **Export Experiment** dialog opens.

3. Name your experiment file and click **Save**.
-

More information

- [Creating a new experiment \(page 29\)](#)
-

Using the bead library

Introduction

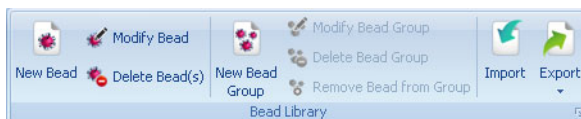
This topic describes how to work with beads and bead groups in the bead library.

Bead files from FCAP Array Version 1.0 cannot be used with Version 3.0. The converse is true also. Bead files from version 3.0 cannot be used with version 1.0.

The bead files for BD CBA reagents can be found at bdbiosciences.com/cbasetup.

Bead functions

All the available beads and bead groups are organized in the bead library. The functions are located in the **Bead Library** group on the ribbon.



Adding beads**To add beads:**

1. Click the **New Bead** icon.

The **New Bead** dialog opens.

2. For each bead, specify the **Bead Name**, **Analyte**, **Second Reporter Parameter** (if applicable), and **Catalog Number** (optional).
 3. Click **OK**.
-

Modifying beads**To modify beads:**

1. Select a bead from the library.
 2. Click the **Modify Bead** icon.
 3. Change the details in the **Modify Bead** dialog.
 4. Click **OK**.
-

Deleting beads**To delete a bead:**

1. Select a bead from the list in the left panel.
2. Click the **Delete Bead** icon.

The **Confirm bead deletion** dialog opens.

3. Click **OK**.
-

Bead group functions

Creating bead groups allow you to manage a set of beads that can be used for specific purposes and makes it more efficient to handle them as a block, rather than individually.

Creating a bead group

To create a bead group:

1. Click the **New Bead Group** icon.
The **New Bead Group** dialog opens.
2. Enter group name and description.
3. Click **OK**.

Modifying a bead group

To modify a bead group:

1. Select a bead group.
2. Click the **Modify Bead Group** icon.
The **Modify Bead Group** dialog opens.
3. Edit the group name and description.
4. Click **OK**.

Deleting a bead group

To delete a bead group:

1. Select a bead group.
2. Click the **Delete Bead Group** icon.
The **Confirm bead group deletion** dialog opens.
3. Click **OK**.

Adding a bead group

To add bead to a group:

1. Select one or more beads from the bead list.
2. Drag and drop them onto the bead group.

Removing a bead from a group

To remove a bead from a group:

1. Select one or more beads from the bead group.
2. Click the **Remove Bead from Group** icon.

Importing a bead group

To import a bead group:

1. Click the **Import** icon.

The **Import** dialog opens.

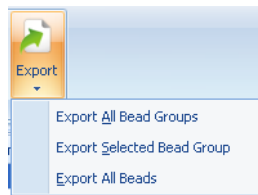
2. Select the XML file you want to import.
 3. Click **Open**.
-

Exporting a bead group

You can export either one single bead group or all groups (including all beads) from the library.

To export a bead group:

1. Select the bead group.
2. Click the **Export** icon and select an export option.



The **Export Bead Data** dialog opens.

3. Enter a file name.
 4. Click **Save**.
-

More information

- [Selecting beads and analysis model \(page 51\)](#)
-

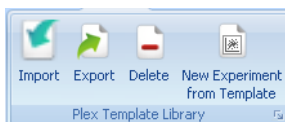
Using the plex template library

Introduction

This topic describes how to work with plex template files in the library. All saved plex templates are stored in the library.

Plex template functions

You can select a plex template to be used in an experiment. The functions are located in the **Plex Template Library** group on the ribbon.



Creating a new experiment from a plex template

To create a new experiment from a plex template:

1. Select a plex template from the plex template table.

Drag a column header here to group by that column			
Name	Creation Date	Created By	Number of Standard Samples
experiment 1	3/18/2011	Administrator	

2. Click the **New Experiment from Template** icon.
The **New Experiment From Template** dialog opens.
3. Enter an experiment name.
4. Click **OK**.

Viewing plex template properties

To view plex template properties:

1. Select a plex from the plex template table.

The properties table displays the details for the selected plex.

Template	
Name	new experiment
Number of Standard Samples	10
Number of Beads	6
Debris Filter	No
Instrument Settings	
Scatter Parameter	SSC-A
Cluster Parameter 1	Red-A
Cluster Parameter 2	NIR-A
Reporter Parameter 1	Yellow-A
Reporter Parameter 2	
Scatter Peaks	1
Fitting	
Fitting Type	5 Parameter Logistic
Weighting	No Weighting
Fitting Accuracy	98.00 %
Force Through Zero	Yes

Renaming a plex template

To rename a plex template:

1. Click in the name field for a template.
2. Edit the name.
3. Press Enter.

Importing a plex template

To import a plex template:

1. Click the **Import** icon.

The **Open** dialog opens.

2. Navigate to your plex template file.
3. Click **Open**.

Exporting a plex template

To export a plex template:

1. Click the **Export** icon.
The **Save As** dialog opens.
2. Enter a name for the plex template.
3. Click **Save**.

Deleting a plex template

To delete a plex template:

1. Select a plex template from the plex template table.
2. Click the **Delete** icon.
The **Confirm template deletion** dialog opens.
3. Click **Yes**.

More information

- [Plex overview \(page 48\)](#)
-

Using the user management library

Introduction This topic describes how to manage users and user privileges.

User management functions Only a user with administrator rights can modify the users list and the rights of users. If the administrator name and password are successfully entered in the **Login** dialog, the user management function is available.

The functions are located in the **User Management** group in the ribbon.



Note that a user with administrator rights can view and edit all experiments.

Adding a new user To add a new user:

1. Click the **New User** icon.

The **New User** dialog opens.

2. Enter details into the fields.

Fields include: **User Name**, **Full Name**, **Department**, **E-mail**, **Contact Info**, **Password**, and **Administrator** checkbox.

Only the **User Name** and **Full Name** are mandatory fields.

3. Click **OK**.

Modifying a user**To modify a user:**

1. Select a user from the user table.

User Name	Full Name	E-Mail
user2		
user3		
Administrator		

2. Click the **Modify User** icon.

The **Modify User** dialog opens.

You can also double-click the user from the user table to open the **Modify User** dialog.

3. Edit user details in the fields.

Fields include: **User Name**, **Full Name**, **Department**, **E-mail**, **Contact Info**, **Password**, and **Administrator** checkbox.

4. Click **OK**.
-

Deleting a user

Note that you cannot delete your own user account and a minimum of one administrator account is required by the software.

To delete a user:

1. Select a user from the user table.
2. Click the **Delete User** icon.

The **Confirm user deletion** dialog opens.

3. Click **Yes**.
-

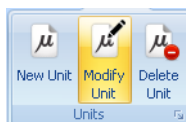
Using the units library

Introduction

This topic describes how to manage the measurement units that are used in experiment analysis when selecting the concentration unit for standard and control samples.

Measurement unit functions

The measurement functions are located in the **Units** group on the ribbon.



Adding a unit

To add a unit:

1. Click the **New Unit** icon.
The **New Unit** dialog opens.
2. Enter the unit name and unit description.
3. Click **OK**.

Modifying a unit

To to modify a unit:

1. Select a unit in the unit table.

Name	Description
ug/mL	
ng/mL	
pg/mL	
fg/mL	
Units/mL	

2. Click the **Modify Unit** icon.
The **Modify Unit** dialog opens.
3. Edit the unit name and unit description.
4. Click **OK**.

Deleting a unit

To delete a unit:

1. Select a unit in the unit table.
2. Click the **Delete Unit** icon.

The **Confirm unit deletion** dialog opens.

3. Click **Yes**.
-

9

Troubleshooting

This chapter covers the following topic:

- [Troubleshooting \(page 116\)](#)

Troubleshooting

Introduction

This section describes tips to help you troubleshoot problems with FCAP Array software.

If you have problems with using FCAP Array, you can find help included in the software. The Help menu is located on the right side of the ribbon.

Calculation error messages

The following table lists calculation error messages in the statistics table.

Message	Description
NaN	Acronym for not a number. Can be the result of zero divided by zero or no calculation done.
negative infinity	Negative number divided by zero.
positive infinity	Positive number divided by zero.

FCAP Array does not start

Possible causes	Recommended solutions
Too many software instances are running on the PC	Close unnecessary applications.
The security key is not connected	Connect the FCAP Array security key to an available USB port on the computer.
Missing program files	Reinstall the software.

User cannot log in

Possible causes	Recommended solutions
User name is incorrect	Check the caps lock key. Re-type your user name.
Forgot user name	Contact your software administrator.
Password is incorrect	Check the caps lock key. Re-type your password.
Forgot password	Your software administrator can reset your password.

User unable to open or edit data

Possible causes	Recommended solutions
Database is corrupted	Restore a database backup or delete the database file and FCAP Array will regenerate the database at the next run.

User manual does not open from Help menu

Possible causes	Recommended solutions
User manual file was not installed with the software	Re-run the FCAP Array installer and select Repair .

Experiment cannot be edited

Possible causes	Recommended solutions
Experiment is locked	Make a copy of the experiment by click the Save As icon in the Experiment group.

New bead cannot be added to the bead library

Possible causes	Recommended solutions
Bead name and analyte already exist in the bead library	Make corrections to the bead identifiers.

Unexpected analysis results

Possible causes	Recommended solutions
Re-using saved fitting curves	Calculate new standard curves for every experiment.
Software extrapolation leads to inaccurate values	Calculate new standard curves for every experiment.
Single-color and dual-color beads are in the same experiment	Single-color and dual-color beads require different instrument setups.
Experiment sample layout does not match actual sample layout	Correct the experiment layout, redo the file assignment, and re-analyze.
Incorrect bead information in the bead library	Correct the information. Import the bead list from an XML file to avoid entry errors.
Incorrect file assignment	Review the acquisition sample order. Correct the file assignment and re-analyze.
Too much debris in one or more samples	Use Debris Filtering to define gated data that excludes debris.

Unable to fit standard curves

Possible causes	Recommended solutions
One or more standard samples failed	Remove bad data points in the Standard Curves window and re-analyze. Acquire another standard sample series.
Standards are fitted out of sequence	Correct the file assignment in the Experiment Design view.
Choice of fitting curve does not model the standard sample data	Choose another fitting curve. 4 Parameter and 5 Parameter Logistic curves are recommended for most beads.
Fitting accuracy is too high	Lower the fitting accuracy value in the Standard Curves window.
Sample preparation error	Prepare and acquire a new standard sample set.
Experiment design does not match the actual layout	Correct the experiment layout, redo the file assignment, and re-analyze.

Cannot analyze data file

Possible causes	Recommended solutions
Corrupt or incorrect FCS file format	Use FCS 2.0 or 3.0 list-mode data files only. Re-export list mode data files from the instrument system in FCS 2.0 or 3.0 format.

During plex definition, clustering fails or incorrect number of clusters are found

Possible causes	Recommended solutions
Data file from the wrong experiment was loaded	Load a data file from the correct experiment.
Wrong scatter or clustering parameters specified	Specify the correct parameters.
Incorrect instrument settings	Correct the instrument settings so that clusters are in the expected locations.
Cluster overlap in data files	Use manual clustering.

More information

- [Technical assistance \(page 9\)](#)
-

10

Reference information

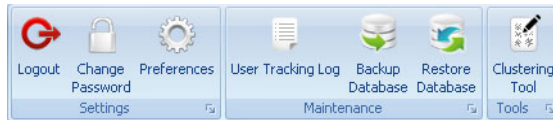
This chapter covers the following topic:

- [Using settings functions \(page 122\)](#)

Using settings functions

Introduction

This topic describes the functions of the **Settings** tab in the main menu. Click the **Settings** tab to display the settings functions.



Logging out

To log out from FCAP Array:

1. Click the **Logout** icon.

The application closes and the **Login** window opens.

Changing your password

When using FCAP Array for the first time, the initial password is created by an administrator. After logging in you can change it at any time.

To change your password:

1. Click the **Change Password** icon.

The **Change Password** dialog opens.

2. Type in your old password and new password.
3. Click **OK**.

Resetting a forgotten password

If you forget your password, an administrator can reset your password.

To reset a user password:

1. Log in as an administrator.
2. Click the **User Management** icon.
3. Double-click a user in the user table.

The **Modify User** dialog opens.

4. Enter a new password for the user.
 5. Click **OK**.
-

Changing user preferences**To change user preferences:**

1. Click the **Preferences** icon.

The **User Preferences** dialog opens.

2. Change the **Default Measurement Unit**, **Default Fitting Accuracy**, or the default **FACSuite Directory** for your BD FACSuite FCS files.
 3. Click **OK**.
-

Viewing the user tracking log**To view the user tracking log:**

1. Click the **User Tracking Log** icon.

The user tracking log shows the following events: software startup, software close, user login, user logout, experiment open, experiment close, experiment create, experiment save, experiment delete, experiment export, and experiment import.

Using the Clustering Test Tool

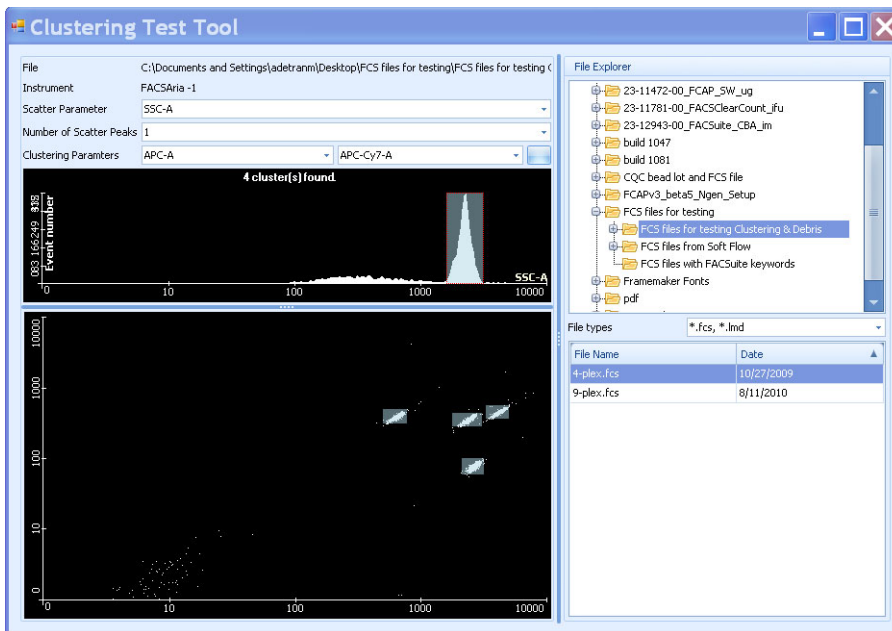
Use this tool to check for correct clustering without needing to go through the plex creation workflow.

To use the Clustering Test Tool:

1. Click the **Clustering Test Tool** icon.

The **Clustering Test Tool** window opens.
2. Navigate to your FCS files in the **File Explorer**.
3. Select your scatter parameter, number of peaks, and cluster parameters.

The autoclustering is shown in the plots.



Backing up the database**To back up the database:**

1. Click the **Backup Database** icon.

The **Save As** dialog opens.

2. Designate a path and filename for your backup file.
3. Click **Save**.

The backup database function does not save your settings. The settings include: last used instrument settings parameters, report header logo images, and FCS data files. To back up your database and settings, go to one of the following folders in Windows and manually make a copy of the FCAP.sdf file.

Windows XP folder: C:\Documents and Settings\All Users\Application Data\Soft Flow\FCAP Array v3

Windows 7 folder: C:\ProgramData\Soft Flow\FCAP Array v3

Restoring the database**To restore the database from a saved database file:**

1. Click the **Restore Database** icon.

The **Open** dialog is displayed.

2. Navigate to the location of your database file.
 3. Click **Open**.
-

Closing FCAP Array**To close FCAP Array software:**

1. Click the exit icon (X) in the top right corner of the application.

If you have an experiment open, you will need to click the exit icon twice.

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