

BD FACSuite™ Software Research Assays Guide

For Research Use Only

bdbiosciences.com
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Becton, Dickinson and Company
BD Biosciences
San Jose, CA 95131
Tel 877.232.8995
Fax 408.954.2347
ResearchApplications@bd.com

BD Biosciences
European Customer Support
Tel 32.2.400.98.95
Fax 32.2.401.70.94
help.biosciences@europe.bd.com

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Regulatory information

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History

| Revision | Date | Change made |
|---------------------|--------|--------------|
| 23-11470-00 Rev. 01 | 7/2011 | New document |

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About this guide

This chapter covers the following topics:

- [About this guide \(page 6\)](#)
- [Technical support \(page 7\)](#)

About this guide

Introduction This topic describes the BD FACSuite™ Software Research Assays Guide. This guide provides instructions for running the BD FACSuite software research assays and shows example lab reports with data for each assay type.

Assumptions This guide assumes that you have read the *BD FACSuite System User's Guide* and that you are familiar with running the software and cytometer.

Additional information See the reagent technical data sheets for instructions on sample preparation and troubleshooting.

In BD FACSuite software, use the Reference menu to:

- Access a PDF of the *BD FACSuite System User's Guide* for instructions on setup, acquisition, and analysis.
 - Open the *BD FACSuite System Reference* that includes additional concepts, procedures, and reference information about the cytometer and BD FACSuite software.
-

Technical support

Introduction This topic describes how to obtain assistance from BD Biosciences technical support.

Before contacting technical support Try the following options for answering technical questions and solving problems:

- Read the section of this guide specific to the operation you are performing.
- Read topics of related information that are listed in the *More Information* section.
- Search the *BD FACSVerse System Reference* for a specific topic.

Contacting technical support **To contact customer support:**

1. Go to bdbiosciences.com.
2. Select your region.
3. Click the **Support** link for details for your local region.

When contacting BD Biosciences, have the following information available:

- System health report that includes the product name, part number, serial number, and details of recent system performance.
- Any error messages.

More information

- [About this guide \(page 6\)](#)

Additional information See topics about generating a system health report in the *BD FACSVerse System Reference*.

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Introduction

This chapter covers the following topic:

- [Overview of the research assays \(page 10\)](#)

Overview of the research assays

Introduction This topic gives a brief overview of the research assays available with BD FACSuite software.

About the research assays The BD FACSuite research assays have been designed to be used with BD flow cytometry reagent kits and the BD FACVerse™ system. The assays provide a quick and easy way to acquire and analyze data. Each assay contains the following elements:

- Pre-defined tubes for samples and controls
- Tube settings and keywords
- Acquisition properties and stopping rules
- Acquisition worksheets with plots and gates
- Analysis worksheets with statistics and annotations
- Reports

Available assays There are nine research assays. The following table shows the predefined tubes in each assay.

| Assay name | Tube 1 | Tube 2 | Tube 3 | Tube 4 |
|------------------------|--------------|-------------|-------------------|-----------------|
| Annexin V FITC | Untreated | Treated | NA | NA |
| Annexin V FITC + block | Untreated | Treated | Untreated + block | Treated + block |
| Annexin V PE | Untreated | Treated | NA | NA |
| Caspase-3 FITC | Untreated | Treated | NA | NA |
| BrdU APC | Unpulsed | Pulsed | NA | NA |
| BrdU FITC | Unpulsed | Pulsed | NA | NA |
| Cycletest | Test sample | DNA control | NA | NA |
| FastImmune CD4 3C | Unstimulated | Positive | Stimulated | NA |
| FastImmune CD4 4C | Unstimulated | Positive | Stimulated | NA |

Assay kits

The following table shows the assay name, corresponding kit name, and kit catalog number.

| Assay name | Assay kit name | Catalog No. |
|------------------------|---|---|
| Annexin V FITC | FITC Annexin V apoptosis detection kit I | 556547 |
| Annexin V FITC + block | FITC Annexin V apoptosis detection kit II | 556570 |
| Annexin V PE | PE Annexin V apoptosis detection kit I | 559763 |
| Caspase-3 FITC | FITC active Caspase-3 apoptosis kit | 550480 |
| BrdU APC | APC BrdU flow kit | 552598 (50 tests) 557892 (4x50 tests) |
| BrdU FITC | FITC BrdU flow kit | 559619 (50 tests) 557891 (4x50 tests) |
| Cycletest | BD Cycletest™ Plus DNA kit | 340242 |
| FastImmune CD4 3C | BD FastImmune™ 3-color CD4 intracellular cytokine detection kit | 340970 (IFN- γ) 340971 (IL-2) 340972 (TNF- α) |
| FastImmune CD4 4C | BD FastImmune™ 4-color CD4 intracellular cytokine detection kit | 337185 (IFN- γ) 337187 (IL-2) 337189 (TNF- α) |

More information

- [Annexin V assays \(page 13\)](#)
- [BrdU assays \(page 33\)](#)
- [Caspase-3 FITC assay \(page 45\)](#)
- [BD Cycletest assay \(page 55\)](#)
- [BD FastImmune assays \(page 69\)](#)

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Annexin V assays

This chapter covers the following topics:

- [About the Annexin V assays \(page 14\)](#)
- [Initial setup of Annexin V assays workflow \(page 15\)](#)
- [Assigning Annexin V FITC and Annexin V PE to the correct detectors \(page 16\)](#)
- [Adding Annexin V FITC and PI to reference settings \(page 17\)](#)
- [Adding Annexin V PE and 7-AAD to reference settings \(page 21\)](#)
- [Annexin V assays workflow \(page 25\)](#)
- [Setting up the software \(page 25\)](#)
- [Running the Annexin V samples \(page 28\)](#)
- [Viewing an Annexin V assay lab report \(page 30\)](#)

About the Annexin V assays

Introduction This topic provides a brief description of the Annexin V assays.

Purpose of the assays The Annexin V assays are used to quantitatively determine the percentage of cells within a population that are actively undergoing apoptosis.

The following table shows the Annexin V assays supported by BD FACSuite software, assay kit catalog numbers, and the fluorochrome and DNA dye used in each assay.

| Assay name | Catalog No. | Fluorochrome | DNA dye |
|-------------------|-------------|--------------|-----------------------|
| Annexin V FITC | 556547 | FITC | Propidium iodide (PI) |
| Annexin V + Block | 556570 | FITC | PI |
| Annexin V PE | 559763 | PE | 7-AAD |

Additional information

See the following technical data sheets:

- *FITC Annexin V Apoptosis Detection Kit I*
 - *FITC Annexin V Apoptosis Detection Kit II*
 - *PE Annexin V Apoptosis Detection Kit I*
-

Initial setup of Annexin V assays workflow

Introduction

This topic describes the basic workflow for initial setup of the Annexin V assays. The workflow varies depending on which assays you will be running. Initially, assign fluorochromes to the correct detector. Add fluorochromes to reference settings for either or both assays. Once complete, repeat these procedures only when you install a new database. You need administrator privileges to perform these procedures.

Workflow

The stages in this workflow include:

| Stage | Description |
|-------|---|
| 1 | Assigning Annexin V FITC and Annexin V PE to the correct detectors (page 16) |
| 2 | Adding Annexin V FITC and PI to reference settings (page 17) or Adding Annexin V PE and 7-AAD to reference settings (page 21) |

Next step

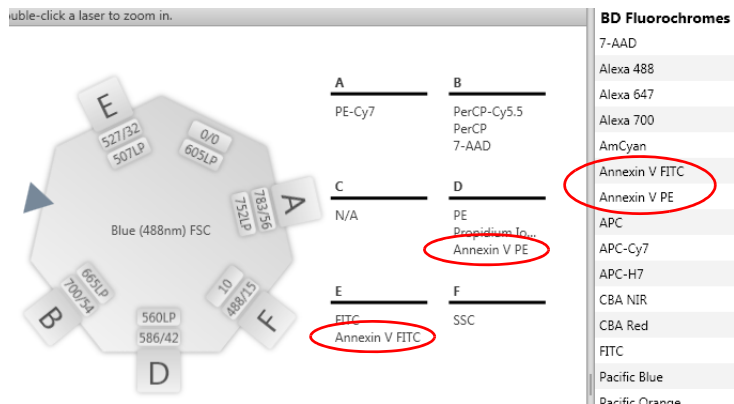
[Assigning Annexin V FITC and Annexin V PE to the correct detectors \(page 16\)](#)

Assigning Annexin V FITC and Annexin V PE to the correct detectors

Introduction This topic describes the steps required to assign Annexin V FITC and Annexin V PE to the correct detectors. You will need administrator privileges to perform this procedure.

Procedure To assign Annexin V FITC and Annexin V PE to the correct detectors:

1. From the **Setup & QC** workspace, click the **Configurations** tab.
2. Double-click the Blue laser configuration to enlarge it.
3. From the **BD Fluorochromes** list:
 - a. Select **Annexin V FITC** and drag it to the bottom of the **E** detector fluorochromes list.
 - b. Select **Annexin V PE** and drag it to the bottom of the **D** detector fluorochromes list.



Next step

- If you will be running the Annexin V FITC or Annexin V + Block assays, go to [Adding Annexin V FITC and PI to reference settings](#) (page 17).
 - If you will be running the Annexin V PE assay, go to [Adding Annexin V PE and 7-AAD to reference settings](#) (page 21).
-

Adding Annexin V FITC and PI to reference settings

Introduction

The topic describes the steps required to add the Annexin V FITC and propidium iodide (PI) fluorochromes to the Lyse Wash reference settings. Perform this procedure if you will be running the Annexin V FITC or Annexin V + Block assays. Once complete, repeat this procedure only when you install a new database. You need administrator privileges to perform this procedure.

Before you begin

- Prepare the BD FACSuite CS&T research beads according to the instructions in the technical data sheet.
 - Prepare three tubes:
 - Unstained sample (negative control)
 - Annexin V FITC-stained sample
Use 1 μL of Annexin V FITC from the FITC Annexin V apoptosis detection kit I to stain the sample.
 - PI-stained sample
Use the PI from the FITC Annexin V apoptosis detection kit I or kit II to stain the sample.
-

Procedure

To add Annexin V FITC and PI to the Lyse Wash reference settings:

1. Create a new experiment with one tube.

2. Right-click the tube in the experiment and select **Add Fluorochromes**.

The **Add Fluorochromes** wizard opens.

3. Select the BD FACSuite CS&T research beads lot number you are running.
4. Under **Reference Settings**, select the checkbox next to the **Lyse Wash** reference settings.

Add Fluorochromes

Select the controls and lot IDs that will be used to create reference settings. Click **Add** or **Delete** to modify which tubes to run.

CS&T

CS&T Bead Lot ID: 92110 (RUO, Expires: 10/1/2020)

Kits

| Kit | Lot ID |
|----------------------|-------------------|
| 4c Research Kit | 101010 (2/1/2012) |
| 4c Plus Research Kit | 101012 (2/1/2012) |
| Violet Research Kit | 101011 (2/1/2012) |

Reference Settings

| Add Fluorochrome(s) | Name | Modified Date | Short descrip |
|-------------------------------------|--------------|----------------------|---------------|
| <input type="checkbox"/> | Lyse No Wash | 7/18/2011 2:34:33 PM | Lyse No Wa |
| <input checked="" type="checkbox"/> | Lyse Wash | 7/18/2011 2:34:33 PM | Lyse Wash |

5. Add **Annexin V FITC**:
 - a. Click **Add** to add a new fluorescence control tube.
 - b. In the **Fluorochrome** column, click the arrow and select **Annexin V FITC** for the new control tube.
 - c. In the **Control Type** column, select **FC** as the control type.
 - d. In the **Unstained** column, select **Unstained (FC)**, which is a separate tube for the unstained control.

Control Tubes

| Fluorochrome | Control Type | Label | Lot ID | Unstained |
|----------------|--------------|---------|--------|----------------|
| Annexin V FITC | FC | Generic | | Unstained (FC) |

6. Add PI:
 - a. Click **Add** to add a new fluorescence control tube.
 - b. In the **Fluorochrome** column, select **Propidium Iodide** for the new control tube.
 - c. In the **Control Type** column, select **FC** as the control type.
 - d. In the **Unstained** column, do not select a control type. Leave the field blank.

7. Click **Next**.

The wizard displays the list of control tubes to acquire. The run pointer is set to the first control tube (CS&T) in the list.

8. Acquire the CS&T tube according to the instructions in the wizard.
 - a. Load a tube of BD FACSuite CS&T research beads on the cytometer.
 - b. Click **Acquire**.

During acquisition, plots display data for each fluorochrome.
 - c. When acquisition of the tube completes, remove the tube.

A green checkmark is displayed next to the CS&T tube indicating that the tube was acquired successfully.

9. Acquire the unstained tube.
 - a. Move the run pointer to the unstained tube.
 - b. Load the unstained tube on the cytometer.
 - c. Click **Acquire**.
 - d. Adjust the gate in the FSC vs SSC plot to enclose the population of interest.
 - e. When acquisition of the tube completes, remove the unstained tube.

10. Acquire the Annexin V FITC tube.
 - a. Move the run pointer to the Annexin V FITC tube.
 - b. Load the Annexin V FITC tube on the cytometer.
 - c. Click **Acquire**.
 - d. Adjust the gate in the FSC vs SSC plot to enclose the population of interest.
 - e. When acquisition of the tube completes, remove the Annexin V FITC tube.
 - f. Ensure that the histogram gate for the positive events is positioned correctly to include the positive population.
11. Repeat [step 10](#) for the Propidium Iodide tube.
12. (Optional) Click **Print** to print the plots for each tube (in a single file) or click **Save as PDF** to save the file.
13. Click **Finish**.

The selected reference settings now include the measured spillover values (SOVs) for the new fluorochromes (DNA dyes).

Next step

[Setting up the software \(page 25\)](#)

Adding Annexin V PE and 7-AAD to reference settings

Introduction

The topic describes the steps required to add the Annexin V PE and 7-AAD fluorochromes to the Lyse Wash reference settings. Perform this procedure if you will be running the Annexin V PE assay. Once complete, repeat this procedure only when you install a new database. You need administrator privileges to perform this procedure.

Before you begin

- Prepare the BD FACSuite CS&T research beads according to the instructions in the technical data sheet.
 - Prepare three tubes:
 - Unstained sample (negative control)
 - Annexin V PE-stained sample
Use the Annexin V PE from the PE Annexin V apoptosis detection kit I to stain the sample.
 - 7-AAD-stained sample
Use the 7-AAD from the PE Annexin V apoptosis detection kit I to stain the sample.
-

Procedure

To add Annexin V PE and 7-AAD to the Lyse Wash reference settings:

1. Create a new experiment with one tube.
2. Right-click the tube in the experiment and select **Add Fluorochromes**.

The **Add Fluorochromes** wizard opens.

3. Select the BD FACSuite CS&T research beads lot number you are running.

4. Under **Reference Settings**, select the checkbox next to the **Lyse Wash** reference settings.

Add Fluorochromes

Select the controls and lot IDs that will be used to create reference settings.
Click **Add** or **Delete** to modify which tubes to run.

CS&T

CS&T Bead Lot ID: 92110 (RUO, Expires: 10/1/2020)

Kits

| Kit | Lot ID |
|----------------------|-------------------|
| 4c Research Kit | 101010 (2/1/2012) |
| 4c Plus Research Kit | 101012 (2/1/2012) |
| Violet Research Kit | 101011 (2/1/2012) |

Reference Settings

| Add Fluorochrome(s) | Name | Modified Date | Short descrip |
|-------------------------------------|--------------|----------------------|---------------|
| <input type="checkbox"/> | Lyse No Wash | 7/18/2011 2:34:33 PM | Lyse No Wa |
| <input checked="" type="checkbox"/> | Lyse Wash | 7/18/2011 2:34:33 PM | Lyse Wash |

5. Add Annexin V PE:
 - a. Click **Add** to add a new fluorescence control tube.
 - b. In the **Fluorochrome** column, click the arrow and select **Annexin V PE** for the new control tube.
 - c. In the **Control Type** column, select **FC** as the control type.
 - d. In the **Unstained** column, select **Unstained (FC)**, which is a separate tube for the unstained control.

Control Tubes

| Fluorochrome | Control Type | Label | Lot ID | Unstained |
|--------------|--------------|---------|--------|-----------|
| Annexin V PE | FC | Generic | | |

6. Add 7-AAD:
 - a. Click **Add** to add a new fluorescence control tube.
 - b. In the **Fluorochrome** column, select **7-AAD** for the new control tube.
 - c. In the **Control Type** column, select **FC** as the control type.

- d. In the **Unstained** column, do not select a control type. Leave the field blank.
7. Click **Next**.

The wizard displays the list of control tubes to acquire. The run pointer is set to the first control tube (CS&T) in the list.
8. Acquire the CS&T tube according to the instructions in the wizard.
 - a. Load a tube of BD FACSuite CS&T research beads on the cytometer.
 - b. Click **Acquire**.

During acquisition, plots display data for each fluorochrome.
 - c. When acquisition of the tube completes, remove the tube.

A green checkmark is displayed next to the CS&T tube indicating that the tube was acquired successfully.
9. Acquire the unstained tube.
 - a. Move the run pointer to the unstained tube.
 - b. Load the unstained tube on the cytometer.
 - c. Click **Acquire**.
 - d. Adjust the gate in the FSC vs SSC plot to enclose the population of interest.
 - e. When acquisition of the tube completes, remove the unstained tube.
 - f. Ensure that the gates for the negative and positive events are positioned correctly to include the correct populations.
10. Acquire the Annexin V PE tube.
 - a. Move the run pointer to the Annexin V PE tube.
 - b. Load the Annexin V PE tube on the cytometer.
 - c. Click **Acquire**.

- d. Adjust the gate in the FSC vs SSC plot to enclose the population of interest.
 - e. When acquisition of the tube completes, remove the Annexin V PE tube.
 - f. Ensure that the histogram gate for the positive events is positioned correctly to include the positive population.
11. Repeat [step 10](#) for the 7-AAD tube.
 12. (Optional) Click **Print** to print the plots for each tube (in a single file) or click **Save as PDF** to save the file.
 13. Click **Finish**.

The selected reference settings now include the measured spillover values (SOVs) for the new fluorochromes (DNA dyes).

Next step

[Setting up the software \(page 25\)](#)

Annexin V assays workflow

Introduction This topic guides you through a typical workflow when running samples stained with one of the Annexin V apoptosis detection kits. See [About the Annexin V assays \(page 14\)](#) for a list of supported assay kits.

Workflow The stages in this workflow include:

| Stage | Description |
|-------|---|
| 1 | Setting up the software (page 25) |
| 2 | Running the Annexin V samples (page 28) |
| 3 | Viewing an Annexin V assay lab report (page 30) |

Setting up the software

Introduction This topic describes how to set up the software to run an Annexin V assay.

- Before you begin**
1. Prepare and stain the samples according to the instructions in the technical data sheet.
 2. If this is the first time you are running the Annexin V assays, follow the instructions in [Assigning Annexin V FITC and Annexin V PE to the correct detectors \(page 16\)](#) for instructions.
 3. Verify that Performance QC was run and that it passed.
 4. Run Assay and Tube Settings Setup for the Annexin V assays and verify that the status is **OK**.

See topic on running assay and tube settings setup in the *BD FACSVerser System User's Guide*.

Procedure

To set up the software:

1. From the Home page, click the **Acquire Data in Worklist** shortcut.

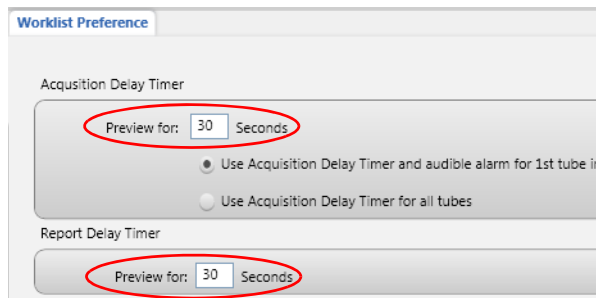


A new worklist opens.

2. From the menu bar, select **Tools > Preferences**.
3. In the **Worklist Preference** tab, set the preview time for the:
 - Acquisition delay timer
 - Report delay timer

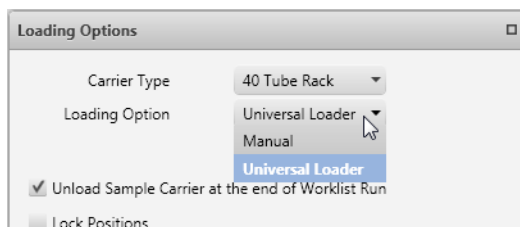
Ensure that the preview time is adequate to review the data and gates before acquisition begins.

Ensure that you have sufficient sample to accommodate increased preview times.

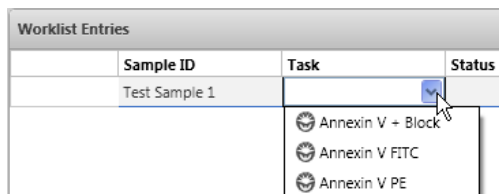


4. Set other worklist preferences as necessary. See topics about setting worklist preferences in the *BD FACSVerser System Reference* for more information.

- Select loading options in the **Loading Options** panel.



- In the worklist, type a sample ID for the first entry and select one of the Annexin V assays from the **Task** menu.



All tubes associated with the assay are added to the entry. This example shows the tubes added for the Annexin V FITC assay.

| Worklist Entries | | | |
|------------------|---------------|----------------|------------------|
| | Sample ID | Task | Status |
| | Test Sample 1 | Annexin V FITC | Ready |
| 1.1 | | Untreated | Ready To Acquire |
| 1.2 | | Treated | Ready To Acquire |

— first tube
— second tube

- Repeat [step 6](#) for the remaining samples.

Next step

[Running the Annexin V samples \(page 28\)](#)

Running the Annexin V samples

Introduction This topic describes how to run samples stained with one of the Annexin V apoptosis detection kits.

Before you begin Set up the software. See [Setting up the software \(page 25\)](#).

Procedure **To run the samples:**

1. In the worklist, make sure that the **Status** column displays *Ready* for all entries and *Ready to Acquire* for all tubes.

| Worklist Entries | | | |
|------------------|---------------|----------------|------------------|
| | Sample ID | Task | Status |
| 1 | Test Sample 1 | Annexin V FITC | Ready |
| 1.1 | | Untreated | Ready To Acquire |
| 1.2 | | Treated | Ready To Acquire |

2. Click **Run All** in the **Worklist Controls** bar.



3. (Optional) In the dialog, save the worklist with a unique name.
4. Load the first Untreated sample on the cytometer using the loading method you selected in [step 5](#) of [Setting up the software \(page 25\)](#).

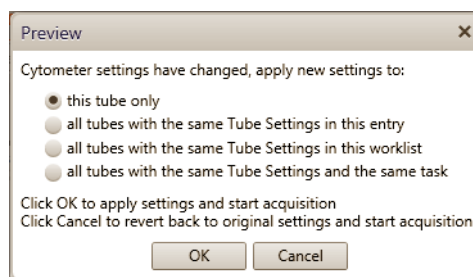
Events are displayed in the plots in the acquisition worksheet.

5. (Optional) Click **Stop Timer** in the **Worklist Controls** bar.

This stops the acquisition delay timer, but sample is still being aspirated into the cytometer.

6. If necessary, adjust:
 - FSC and SSC voltages to bring the population of interest into the Cells gate.
Alternatively, modify the Cells gate to enclose the population of interest.
 - Annexin V vs DNA dye (PI, in this example) voltages to move the populations of interest into the pre-defined gates.
7. Once settings are adjusted, click **Resume** to start acquisition.

The following dialog opens.



8. Select the option that you want to apply and then click **OK**.

The lab report is displayed after all tubes of the first entry have been acquired.

9. (Optional) Click **Stop Timer** to view the lab report plots and make adjustments to the gates, if necessary.
10. Click **Resume** to continue to the next entry.

Next step

[Viewing an Annexin V assay lab report](#)

Additional information

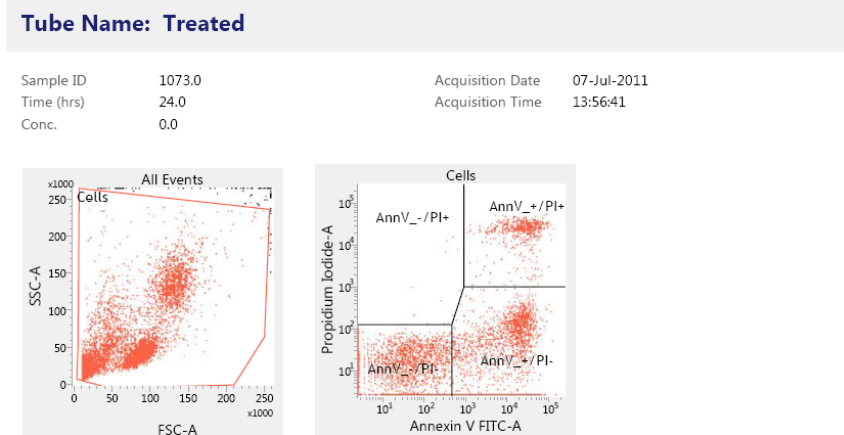
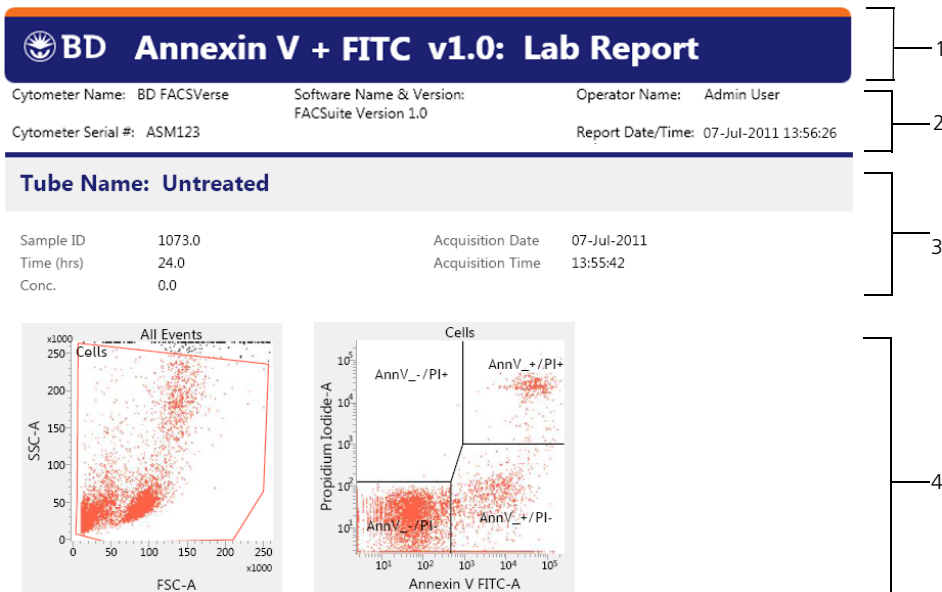
In the *BD FACSVerser System User's Guide*, see topics about running assay and tube settings setup and building a new worklist.

Viewing an Annexin V assay lab report

Introduction

This topic shows an example lab report for the Annexin V assay and describes the elements in the report.

Example lab report



Cytometer Name: BD FACSVersé Software Name & Version: FACSuite Version 1.0 Operator Name: Admin User
 Cytometer Serial #: ASM123 Report Date/Time: 07-Jul-2011 13:56:26

| Results Summary | | | |
|------------------|-----------|---------|---|
| Label | Untreated | Treated | |
| Events Acquired | 10000.0 | 10000.0 | 5 |
| % Cells | 97.7 | 98.3 | |
| % Annexin V-/PI- | 81.8 | 66.2 | |
| % Annexin V+/PI- | 13.9 | 27.3 | |
| % Annexin V+/PI+ | 4.3 | 6.4 | |
| % Annexin V-/PI+ | 0.0 | 0.1 | |

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| No. | Lab report element |
|-----|--|
| 1 | BD banner. Lists the BD logo, assay name, software version number, and report type. |
| 2 | Report header. Lists the cytometer name and serial number, software name and version number, operator name, and date and time the report was generated. |
| 3 | Acquisition information. Displays the tube name, sample ID, acquisition date and time, and keywords. |
| 4 | Acquisition plots. Displays the plots with gates. |
| 5 | Results summary table. Shows the number of events acquired and population percentages for each gate. |
| 6 | Report footer. Displays the regulatory status of the assay and the page number of the report. |

Troubleshooting information

See the troubleshooting sections in the *BD FACSVersé System User's Guide* and the *BD FACSVersé System Reference*.

More information

- [About the Annexin V assays \(page 14\)](#)

4

BrdU assays

This chapter covers the following topics:

- [About the BrdU assays \(page 34\)](#)
- [Assigning 7-AAD \(DNA\) to the correct detector \(page 34\)](#)
- [BrdU assays workflow \(page 35\)](#)
- [Setting up the software \(page 36\)](#)
- [Running the samples \(page 38\)](#)
- [Viewing a BrdU assay lab report \(page 41\)](#)

About the BrdU assays

Introduction This topic provides a brief description of the BrdU assays.

Purpose of the assays The BrdU assays measure incorporated BrdU to identify cells that have synthesized DNA.

The following table shows the BrdU assays supported by BD FACSuite software, assay kit catalog numbers, and the fluorochrome and DNA dye used in each assay.

| Assay name | Catalog No. | Fluorochrome | DNA dye |
|------------|--|--------------|---------|
| BrdU APC | <ul style="list-style-type: none"> • 552598 (50 tests) • 557892 (4x50 tests) | APC | 7-AAD |
| BrdU FITC | <ul style="list-style-type: none"> • 559619 (50 tests) • 557891 (4x50 tests) | FITC | 7-AAD |

Additional information See the *BrdU Flow Kits* instruction manual.

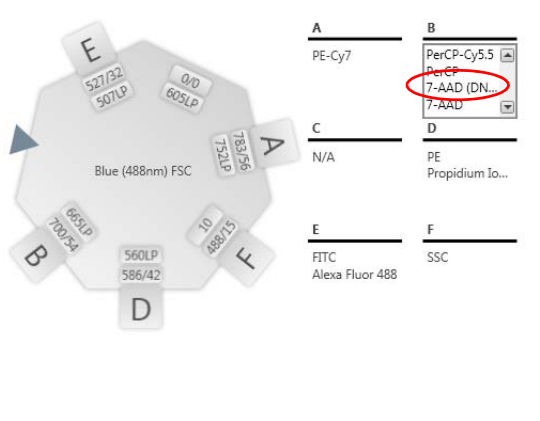
Assigning 7-AAD (DNA) to the correct detector

Introduction This topic describes the steps required to assign 7-AAD (DNA) to the correct detector. You need to perform this procedure before running one of the BrdU assays. Once complete, repeat this procedure only when you install a new database. You will need administrator privileges to perform this procedure.

Procedure To assign the 7-AAD (DNA) to the correct detector:

1. From the **Setup & QC** workspace, click the **Configurations** tab.
2. Double-click the Blue laser configuration to enlarge it.

- From the **BD Fluorochromes** list, select **7-AAD (DNA)** and drag it to the bottom of the **B** detector fluorochromes list.



Next step

[Setting up the software \(page 36\)](#)

BrdU assays workflow

Introduction

This topic guides you through a typical workflow when running samples stained with the BrdU APC or BrdU FITC assay kit. See [About the BrdU assays \(page 34\)](#) for a list of supported assay kits.

Workflow

The stages in this workflow include:

| Stage | Description |
|-------|---|
| 1 | Setting up the software (page 36) |
| 2 | Running the samples (page 38) |
| 3 | Viewing a BrdU assay lab report (page 41) |

Setting up the software

Introduction

This topic describes how to set up the software to run either the BrdU APC or BrdU FITC assay.

Before you begin

1. Prepare and stain the samples according to the instructions in the *BrdU Flow Kits* instruction manual for FITC and APC.
2. Verify that Performance QC was run and that it passed.
3. Run Assay and Tube Settings Setup for the BrdU assays and verify that the status is **OK**.

See Running Assay and Tube Settings Setup in the *BD FACSVerser System User's Guide*.

4. Assign 7-AAD (DNA) to the correct detector. See [Assigning 7-AAD \(DNA\) to the correct detector \(page 34\)](#) for instructions.

Procedure

To set up the software:

1. From the Home page, click the **Acquire Data in Worklist** shortcut.



A new worklist opens.

2. From the menu bar, select **Tools > Preferences**.
3. In the **Worklist Preference** tab, set the preview time for the:
 - Acquisition delay timer
 - Report delay timer

Ensure that the preview time is adequate to review the data and gates before acquisition begins.

Ensure that you have sufficient sample to accommodate increased preview times.

Worklist Preference

Acquisition Delay Timer

Preview for: 30 Seconds

Use Acquisition Delay Timer and audible alarm for 1st tube in
 Use Acquisition Delay Timer for all tubes

Report Delay Timer

Preview for: 30 Seconds

4. Set other worklist preferences as necessary. See topics about setting worklist preferences in the *BD FACSVerser System Reference* for more information.
5. Select loading options in the **Loading Options** panel.

Loading Options

Carrier Type: 40 Tube Rack

Loading Option: Universal Loader

Unload Sample Carrier at the end of Worklist Run
 Lock Positions

6. In the worklist, type a sample ID for the first entry and select either the **BrdU APC** or **BrdU FITC** assay from the **Task** menu.

| Worklist Entries | | | |
|------------------|---------------|---|--------|
| | Sample ID | Task | Status |
| | Test Sample 1 | <div style="border: 1px solid black; padding: 2px;"> BrdU APC BrdU FITC </div> | |

All tubes associated with the assay are added to the entry. This example shows the tubes added for the BrdU FITC assay.

| Worklist Entries | | |
|------------------|---------------|-----------|
| | Sample ID | Task |
| 1 | Test Sample 1 | BrdU FITC |
| 1.1 | | Unpulsed |
| 1.2 | | Pulsed |

- Repeat [step 6](#) for the remaining samples.

Next step

[Running the samples \(page 38\)](#)

Running the samples

Introduction

This topic describes how to run samples stained with either the BrdU APC or BrdU FITC assay

Before you begin

Set up the software. See [Setting up the software \(page 36\)](#)

Procedure

To run the samples:

- In the worklist, make sure that the **Status** column displays *Ready* for all entries and *Ready to Acquire* for all tubes.

| Worklist Entries | | | |
|------------------|---------------|-----------|------------------|
| | Sample ID | Task | Status |
| 1 | Test Sample 1 | BrdU FITC | Ready |
| 1.1 | | Unpulsed | Ready To Acquire |
| 1.2 | | Pulsed | Ready To Acquire |

2. Click **Run All** in the **Worklist Controls** bar.



3. (Optional) In the dialog, save the worklist with a unique name.
4. Load the first Unpulsed sample on the cytometer using the loading method you selected in [step 5 of Setting up the software \(page 36\)](#).

Events are displayed in the plots in the acquisition worksheet.

5. (Optional) Click **Stop Timer** in the **Worklist Controls** bar.

This stops the acquisition delay timer, but sample is still being aspirated into the cytometer.

6. If necessary, adjust:

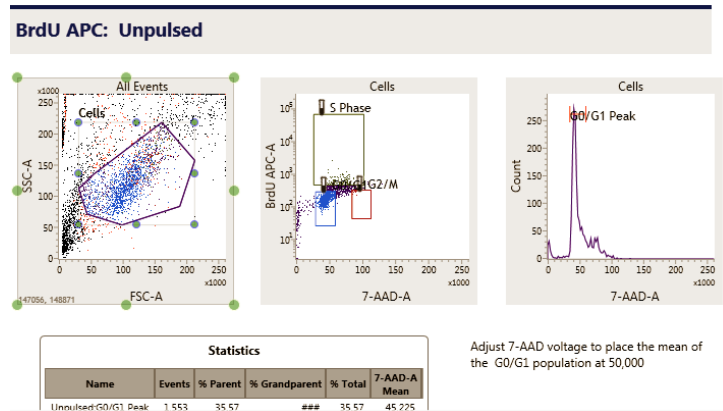
- FSC and SSC voltages to bring the population of interest into the Cells gate.

Alternatively, modify the Cells gates to enclose the population of interest.

- 7-AAD-A voltage to place the mean of the G0/G1 peak in the histogram at 50,000.

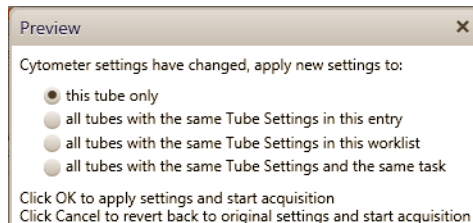
7. If necessary, adjust the 7-AAD-A and BrdU-A voltages to bring the populations of interest into the individual gates.

Alternatively, adjust the S Phase, G0/G1, and G2/M gates in the BrdU vs 7-AAD dot plot to enclose the populations of interest.



- Once settings are adjusted, click **Resume** to start acquisition.

The following dialog opens.



- Select the option that you want to apply and then click **OK**.

The lab report is displayed after all tubes of the first entry have been acquired.

- (Optional) Click **Stop Timer** to view the lab report plots and make adjustments to the gates, if necessary.
- Click **Resume** to continue to the next entry.

Next step

[Viewing a BrdU assay lab report \(page 41\)](#)

Additional information

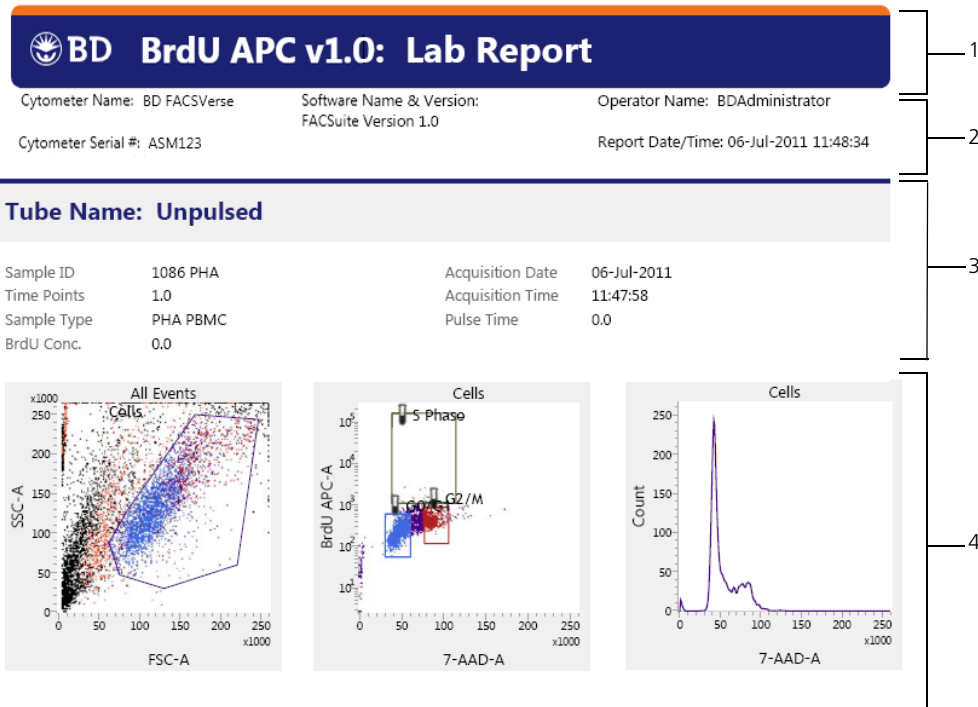
In the *BD FACSVerser System User's Guide*, see topics on running assay and tube settings setup and building a new worklist.

Viewing a BrdU assay lab report

Introduction

This topic shows an example lab report for the BrdU assay and describes the elements in the report.

Example lab report



Cytometer Name: BD FACSVe

Software Name & Version:
FACSuite Version 1.0

Operator Name: BDAAdministrator

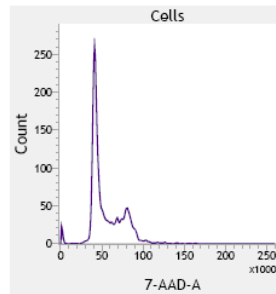
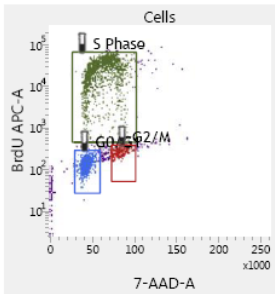
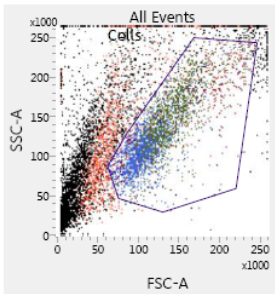
Cytometer Serial #: ASM123

Report Date/Time: 06-Jul-2011 11:48:34

Tube Name: Pulsed

Sample ID 1086 PHA
Time Points 1.0
Sample Type PHA PBMC
BrdU Conc. 50.0

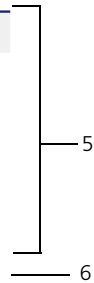
Acquisition Date 06-Jul-2011
Acquisition Time 11:49:33
Pulse Time 1.0



Results Summary

| Label | Unpulsed | Pulsed |
|-------------------|----------|---------|
| Events Acquired | 10000.0 | 10000.0 |
| % Cells | 35.1 | 34.7 |
| % G0/G1 | 66.9 | 44.8 |
| % S Phase (BrdU+) | 0.5 | 39.1 |
| % G2/M | 15.8 | 8.5 |

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| No. | Element |
|-----|--|
| 1 | BD banner. Lists the BD logo, assay name, software version number, and report type. |
| 2 | Report header. Lists the cytometer name and serial number, software name and version number, operator name, and date and time the report was generated. |
| 3 | Acquisition information. Displays the tube name, sample ID, and acquisition date and time. |

| No. | Element |
|-----|---|
| 4 | Acquisition plots. Displays the plots with gates. |
| 5 | Results summary table. Shows the number of events acquired and population percentages for each gate. |
| 6 | Report footer. Displays the regulatory status of the software and the page number of the report. |

Troubleshooting information

See the troubleshooting sections in the *BD FACSVerser System User's Guide* and the *BD FACSVerser System Reference*.

More information

- [About the BrdU assays \(page 34\)](#)
-

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Caspase-3 FITC assay

This chapter covers the following topics:

- [About the Caspase-3 FITC assay \(page 46\)](#)
- [Caspase-3 FITC assay workflow \(page 46\)](#)
- [Setting up the software \(page 47\)](#)
- [Running the samples \(page 50\)](#)
- [Viewing a Caspase-3 FITC assay lab report \(page 52\)](#)

About the Caspase-3 FITC assay

Introduction This topic provides a brief description of the Caspase-3 FITC assay.

Purpose of the assay The Caspase-3 FITC assay measures Caspase-3 activity. Caspase-3 activation is an early marker of cells undergoing apoptosis.

The following table shows the Caspase-3 FITC assay supported by BD FACSuite software, the assay kit catalog number, and the fluorochrome used in the assay.

| Assay name | Catalog No. | Fluorochrome |
|----------------|-------------|--------------|
| Caspase-3 FITC | 550480 | FITC |

Additional information See the *FITC Active Caspase-3 Apoptosis Kit* technical data sheet.

Caspase-3 FITC assay workflow

Introduction This topic guides you through a typical workflow when running samples stained with the FITC Active Caspase-3 apoptosis kit. See [About the Caspase-3 FITC assay \(page 46\)](#) for the supported assay kit.

Workflow

The stages in this workflow include:

| Stage | Description |
|-------|---|
| 1 | Setting up the software (page 47) |
| 2 | Running the samples (page 50) |
| 3 | Viewing a Caspase-3 FITC assay lab report (page 52) |

Setting up the software

Introduction

This topic describes how to set up the software to run the Caspase-3 FITC assay.

Before you begin

1. Prepare and stain the samples according to the instructions in the technical data sheet.
2. Verify that Performance QC was run and that it passed.
3. Run Assay and Tube Settings Setup for the Caspase-3 FITC assay and verify that the status is **OK**.

See topics about running assay and tube settings setup in the *BD FACSVerser System User's Guide*.

Procedure

To set up the software:

1. From the Home page, click the **Acquire Data in Worklist** shortcut.

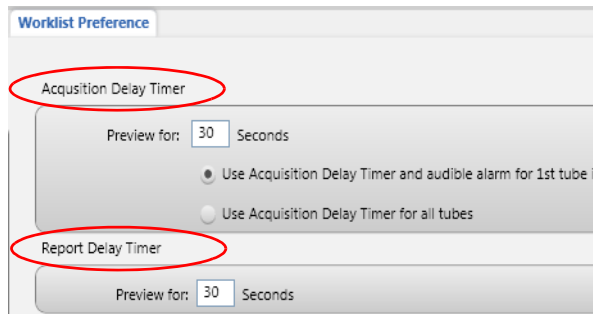


A new worklist opens.

2. From the menu bar, select **Tools > Preferences**.
3. In the **Worklist Preference** tab, set the preview time for the:
 - Acquisition delay timer
 - Report delay timer

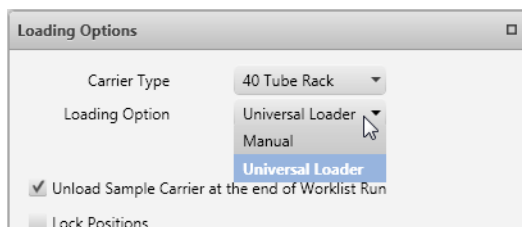
Ensure that the preview time is adequate to review the data and gates before acquisition begins.

Ensure that you have sufficient sample to accommodate increased preview times.

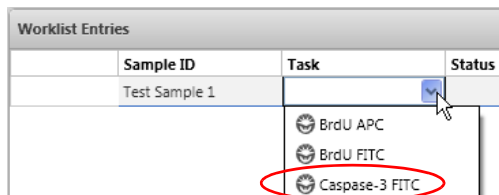


4. Set other worklist preferences as necessary. See topics about setting worklist preferences in the *BD FACSVerser System Reference* for more information.

5. Select loading options in the **Loading Options** panel.



6. In the worklist, type a sample ID for the first entry and select the Caspase-3 FITC assay from the **Task** menu.



All tubes associated with the assay are added to the entry.

| Worklist Entries | | |
|------------------|---------------|----------------|
| | Sample ID | Task |
| 1 | Test Sample 1 | Caspase-3 FITC |
| 1.1 | | Untreated |
| 1.2 | | Treated |

7. Repeat [step 6](#) for the remaining samples.

Next step

[Running the samples \(page 50\)](#)

Running the samples

Introduction This topic describes how to run samples stained with the FITC Active Caspase-3 apoptosis kit.

Before you begin Set up the software. See [Setting up the software \(page 47\)](#).

Procedure **To run the samples:**

1. In the worklist, make sure that the **Status** column displays *Ready* for all entries and *Ready to Acquire* for all tubes.

| Worklist Entries | | | |
|------------------|---------------|----------------|------------------|
| | Sample ID | Task | Status |
| 1 | Test Sample 1 | Caspase-3 FITC | Ready |
| 1.1 | | Untreated | Ready To Acquire |
| 1.2 | | Treated | Ready To Acquire |

2. Click **Run All** in the **Worklist Controls** bar.



3. (Optional) In the dialog, save the worklist with a unique name.
4. Load the first Untreated sample on the cytometer using the loading method you selected in [step 5 of Setting up the software \(page 47\)](#).

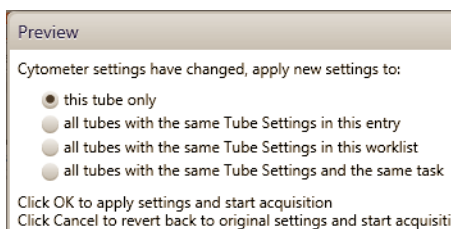
Events are displayed in the plots in the acquisition worksheet.

5. (Optional) Click **Stop Timer** in the **Worklist Controls** bar.

This stops the acquisition delay timer, but sample is still being aspirated into the cytometer.

6. If necessary, adjust:
 - FSC and SSC voltages to bring the population of interest into the Cells gate.
Alternatively, modify the Cells gate to enclose the population of interest.
 - FITC voltage to bring the population of interest into the Active Caspase-3 gate.
Alternatively, modify the gates to enclose the population of interest.
7. Once settings are adjusted, click **Resume** to start acquisition.

The following dialog opens.



8. Select the option that you want to apply and then click **OK**.

The lab report is displayed after all tubes of the first entry have been acquired.
9. (Optional) Click **Stop Timer** to view the lab report plots and make adjustments to the gates, if necessary.
10. Click **Resume** to continue to the next entry.

Next step

[Viewing a Caspase-3 FITC assay lab report \(page 52\)](#)

Additional information

In the *BD FACSVerser System User's Guide*, see topics about running assay and tube settings setup and building a new worklist.

Viewing a Caspase-3 FITC assay lab report

Introduction

This topic shows an example lab report for the Caspase-3 assay and describes the elements in the report.

Example lab report

Caspase-3 FITC v1.0: Lab Report

| | | |
|-----------------------------|---|--|
| Cytometer Name: BD FACSVers | Software Name & Version: FACSuite Version 1.0 | Operator Name: Admin User |
| Cytometer Serial #: ASM123 | | Report Date/Time: 07-Jul-2011 14:50:15 |

Tube Name: Untreated

| | | |
|--------------------------|-------------------------------|--|
| Sample ID: 2079.0 | Acquisition Date: 07-Jul-2011 | |
| Events Acquired: 10000.0 | Acquisition Time: 14:50:15 | |
| Conc.: 0.0 | Time (hrs): 24.0 | |

Tube Name: Treated

| | | |
|--------------------------|-------------------------------|--|
| Sample ID: 2079.0 | Acquisition Date: 07-Jul-2011 | |
| Events Acquired: 10000.0 | Acquisition Time: 14:50:39 | |
| Conc.: 0.0 | Time (hrs): 24.0 | |

Cytometer Name: BD FACSVersé Software Name & Version: FACSuite Version 1.0 Operator Name: Admin User
 Cytometer Serial #: ASM123 Report Date/Time: 07-Jul-2011 14:50:15

| Label | Results |
|---------------------------------------|---------|
| % Positive Caspase-3 cells: Untreated | 4.84 |
| % Positive Caspase-3 cells: Treated | 4.89 |

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6

| No. | Lab report element |
|-----|--|
| 1 | BD banner. Lists the BD logo, assay name, software version number, and report type. |
| 2 | Report header. Lists the cytometer name and serial number, software name and version number, operator name, and date and time the report was generated. |
| 3 | Acquisition information. Displays the tube name, sample ID, and acquisition date and time. |
| 4 | Acquisition plots. Displays the plots with gates. |
| 5 | Results summary table. Shows the number of events acquired and population percentages for each gate. |
| 6 | Report footer. Displays the regulatory status of the assay and the page number of the report. |

More information

- [About the Caspase-3 FITC assay \(page 46\)](#)

Troubleshooting information

See the troubleshooting sections in the *BD FACSVersé System User's Guide* and the *BD FACSVersé System Reference*.

6

BD Cycletest assay

This chapter covers the following topics:

- [About the BD Cycletest assay \(page 56\)](#)
- [BD Cycletest assay workflow \(page 57\)](#)
- [Running the BD DNA QC particles \(page 57\)](#)
- [Setting up the software for the BD Cycletest assay \(page 60\)](#)
- [Running the samples for the BD Cycletest assay \(page 62\)](#)
- [Viewing a BD Cycletest assay lab report \(page 65\)](#)

About the BD Cycletest assay

Introduction This topic provides a brief description of the BD Cycletest assay.

Purpose of the assays The BD Cycletest assay, in combination with the BD Cycletest Plus DNA reagent kit and BD DNA QC particles kit, can be used to estimate cell-cycle phase distribution. The BD Cycletest assay can facilitate in generating a user-defined assay that can be used to identify abnormal DNA stemlines.

To obtain accurate cell cycle phase percentages, we also recommend using a software tool, such as ModFit LT™. ModFit LT software uses mathematical models to classify the phase of cells within the population, and enables analysis of samples which contain complex DNA patterns, including multiple cycles.

The following table shows the assay kits that can be used with the BD Cycletest assay.

| Assay name | Assay kit name | Catalog No. |
|--------------|-----------------------------------|-------------|
| BD Cycletest | BD Cycletest Plus DNA reagent kit | 340242 |
| CEN | BD DNA QC particles kit | 349523 |
| CTN | | |

Additional information

See the following technical data sheets:

- *BD Cycletest Plus DNA Reagent kit*
 - *BD DNA QC particles kit*
-

BD Cycletest assay workflow

Introduction This topic guides you through a typical workflow for setting up the cytometer with BD DNA QC particles and running samples stained with the BD Cycletest kit.

Workflow The stages in this workflow include:

| Stage | Description |
|-------|--|
| 1 | Running the BD DNA QC particles (page 57) |
| 2 | Setting up the software for the BD Cycletest assay (page 60) |
| 3 | Running the samples for the BD Cycletest assay (page 62) |
| 4 | Viewing a BD Cycletest assay lab report (page 65) |

Running the BD DNA QC particles

Introduction This topic describes how to run BD DNA QC particles to set up the BD FACSVerser flow cytometer for DNA analysis. Perform this procedure before running samples stained with the BD Cycletest Plus DNA reagent kit.

- Before you begin**
1. Prepare and stain the CEN and CTN according to the instructions in the *BD DNA QC particles* technical data sheet.
 2. Verify that Performance QC was run and that it passed.
 3. Run Assay and Tube Settings Setup for the BD Cycletest assay and verify that status is **OK**.

See Running Assay and Tube Settings Setup in the *BD FACSVerser System User's Guide* for instructions.

Setting up the software**To set up the software:**

1. Create a new experiment with two tubes.

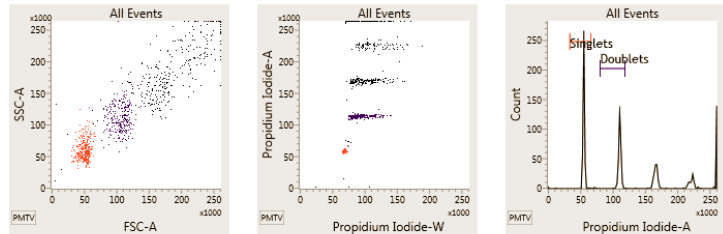
See Building Experiments in the *BD FACSVerse System User's Guide*.
 2. Name the first tube *CEN* and the second tube *CTN*.
 3. In the Data Sources panel, set the run pointer to the CEN tube.
 4. In the CEN and CTN worksheets, create the following:
 - FSC vs SSC dot plot
 - Propidium Iodide-W vs Propidium Iodide-A dot plot with linear scale axes
 - Propidium Iodide-A histogram (with linear axis with two interval gates named *Singlets* and *Doublets*)
 - Statistics view showing Events, % Parent, % Total, Propidium Iodide-A Mean, Propidium Iodide-A CV.
 - Create an expression to calculate the Propidium Iodide-A ratio of the mean of doublets to singlets.
-

Running the samples**To run the samples:**

1. Load the CEN tube on the cytometer using your preferred loading method.
2. Click **Preview**.

Events are displayed in the plots in the worksheet.

- Adjust the propidium-iodide-A voltage to place the mean of the singlet peak at 50,000.



| CEN | | | | | | |
|--------------|--------|----------|---------------|---------|-------------------------|-----------------------|
| Name | Events | % Parent | % Grandparent | % Total | Propidium Iodide-A Mean | Propidium Iodide-A CV |
| CEN:Singlets | 357 | 35.70 | ### | 35.70 | 56.324 | 1.97 |
| CEN:Doublets | 266 | 26.60 | ### | 26.60 | 111.762 | 1.67 |

- Verify that the:
 - CV of the singlet peak is $\leq 3.00\%$.
 - Ratio of the mean of the doublets to singlets is between 1.95 and 2.05.
- Once settings have been adjusted, click **Acquire**.
- When acquisition is complete, click **Next** to move the run pointer to the CTN tube.
- Load the CTN tube on the cytometer using your preferred loading method.
- Repeat [step 2](#), [step 3](#), and [step 5](#) for the CTN tube.
- Save the experiment as a user-defined assay and name it *DNA QC*.

See topics on creating a user-defined assay from an experiment in the *BD FACSVerser System User's Guide* for instructions.

Use the DNA QC assay to run BD DNA QC particles for future setup of the BD Cycletest assay.

-
- More information**
- [Viewing a BD Cycletest assay lab report \(page 65\)](#)
-

Setting up the software for the BD Cycletest assay

Introduction This topic describes how to set up the software to run a BD Cycletest assay.

-
- Before you begin**
1. Set up the cytometer using the BD DNA QC particles. See [Running the BD DNA QC particles \(page 57\)](#).
-

- Procedure**
- To set up the software:**
1. From the Home page, click the **Acquire Data in Worklist** shortcut.



A new worklist opens.

2. From the menu bar, select **Tools > Preferences**.
3. In the **Worklist Preference** tab, set the preview time for the:
 - Acquisition delay timer
 - Report delay timer

Ensure that the preview time is adequate to review the data and gates before acquisition begins.

Ensure that you have sufficient sample to accommodate increased preview times.

Worklist Preference

Acquisition Delay Timer

Preview for: 30 Seconds

Use Acquisition Delay Timer and audible alarm for 1st tube in
 Use Acquisition Delay Timer for all tubes

Report Delay Timer

Preview for: 30 Seconds

4. Set other worklist preferences as necessary. See topics about setting worklist preferences in the *BD FACSVerse System Reference* for more information.
5. Select loading options in the **Loading Options** panel.

Loading Options

Carrier Type: 40 Tube Rack




Loading Option: Universal Loader

Unload Sample Carrier at the end of Worklist Run
 Lock Positions

6. In the worklist, type a sample ID for the first entry and select the BD Cycletest assay from the **Task** menu.

| Worklist Entries | | | |
|------------------|---------------|-----------|--------|
| | Sample ID | Task | Status |
| 1 | Test Sample 1 | Cycletest | |

All tubes associated with the assay are added to the entry.

| Worklist Entries | | |
|------------------|---------------|---|
| | Sample ID | Task |
| ← 1 | Test Sample 1 |  Cycletest |
| 1.1 | |  Test Sample |
| 1.2 | |  DNA Control |

- Repeat [step 6](#) for the remaining samples.

Next step

[Running the samples for the BD Cycletest assay \(page 62\)](#)

Running the samples for the BD Cycletest assay

Introduction

This topic describes how to run samples stained with BD Cycletest Plus DNA reagent kit.

Before you begin

- Prepare and stain a test sample and a control for each entry according to the instructions in the technical data sheet.

If you are using the results of this assay to calculate DNA ploidy, use the test sample spiked with another sample that contains predominantly diploid cells (ie, PBMCs) as the DNA Control.

- Set up the software. See [Setting up the software for the BD Cycletest assay \(page 60\)](#).

Procedure

To run the samples:

1. In the worklist, make sure that the **Status** column displays *Ready* for all entries and *Ready to Acquire* for all tubes.

| Worklist Entries | | | |
|------------------|---------------|-------------|------------------|
| | Sample ID | Task | Status |
| 1 | Test Sample 1 | Cycletest | Ready |
| 1.1 | | Test Sample | Ready To Acquire |
| 1.2 | | DNA Control | Ready To Acquire |

2. Click **Run All** in the **Worklist Controls** bar.



3. (Optional) In the dialog, save the worklist with a unique name.
4. Load the first Test Sample on the cytometer using the loading method you selected in [step 5 of Setting up the software for the BD Cycletest assay \(page 60\)](#).

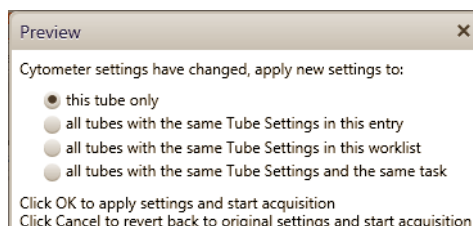
Events are displayed in the plots in the acquisition worksheet.

5. (Optional) Click **Stop Timer** in the **Worklist Controls** bar.

This stops the acquisition delay timer, but sample is still being aspirated into the cytometer.

6. If necessary, adjust the propidium-iodide-A voltage to place the mean of the G_0/G_1 peak at 50,000.
7. Once settings are adjusted, click **Resume** to start acquisition.

The following dialog opens.



8. Select the option that you want to apply and then click **OK**.

The lab report is displayed after all tubes of the first entry have been acquired.

9. (Optional) Click **Stop Timer** to view the lab report plots and make adjustments to the gates, if necessary.
10. Click **Resume** to continue to the next entry.

Next step

[Viewing a BD Cycletest assay lab report](#)

Additional information

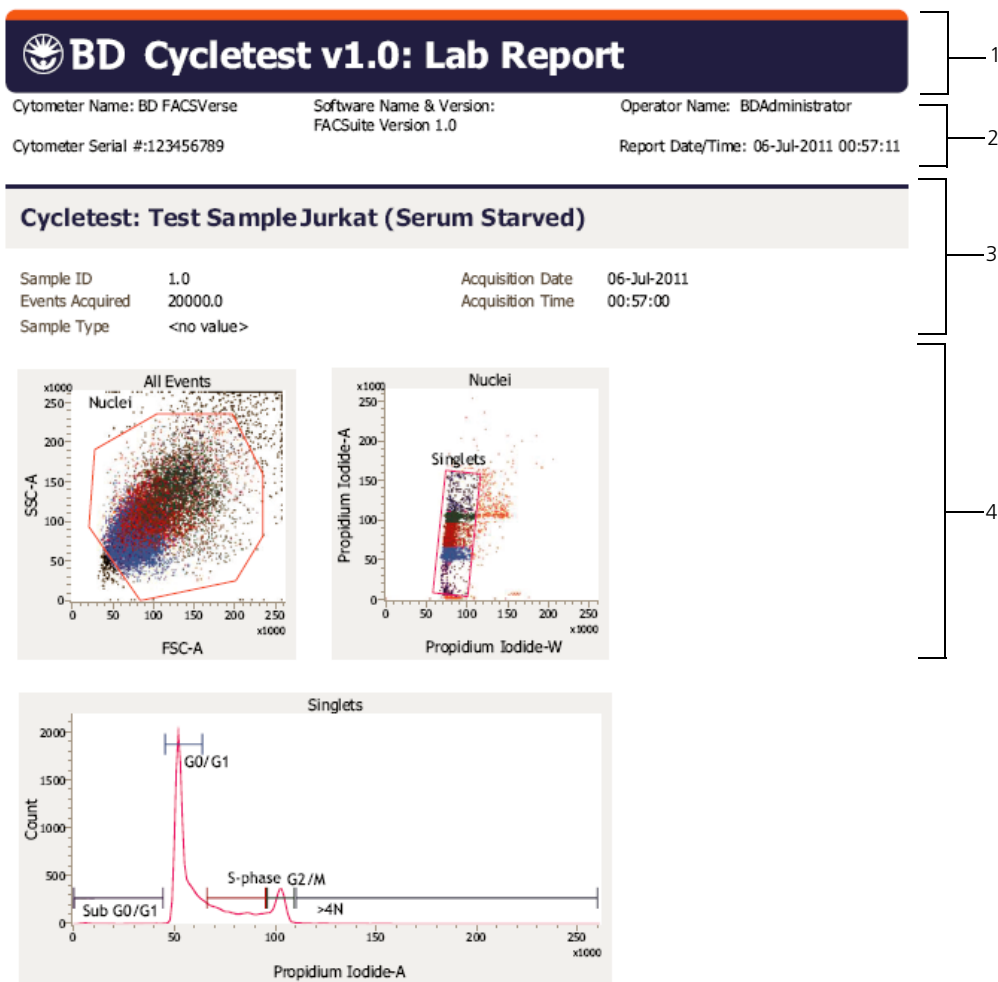
In the *BD FACSVerser System User's Guide*, see topics about running assay and tube settings setup and building a new worklist.

Viewing a BD Cycletest assay lab report

Introduction

This topic shows an example lab report for the BD Cycletest assay and describes the elements in the report.

Example lab report



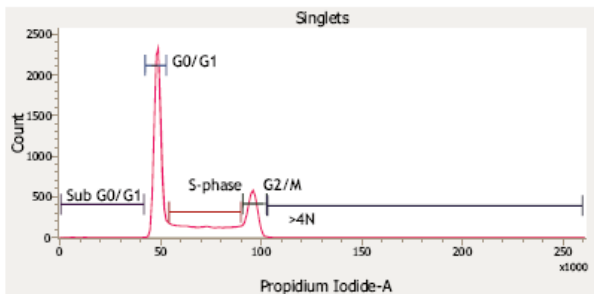
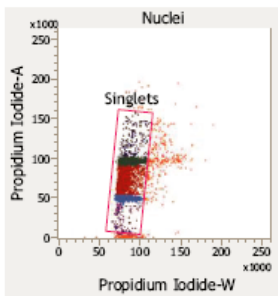
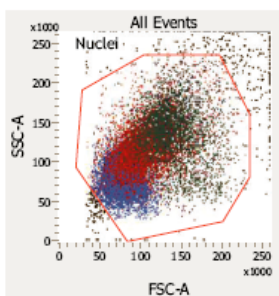
Cytometer Name: BD FACSVersé
 Cytometer Serial #:123456789

Software Name & Version:
 FACSuite Version 1.0

Operator Name: BDAdministrator
 Report Date/Time: 06-Jul-2011 00:57:11

Cycletest: DNA Control Jurkat (Control)

| | | | |
|-----------------|------------|------------------|-------------|
| Sample ID | 1.0 | Acquisition Date | 06-Jul-2011 |
| Events Acquired | 20000.0 | Acquisition Time | 00:57:32 |
| Sample Type | <no value> | | |



Cytometer Name: BD FACSVersé
Cytometer Serial #:123456789

Software Name & Version:
FACSuite Version 1.0

Operator Name: BDAdministrator
Report Date/Time: 06-Jul-2011 00:57:11

| Results Summary | | |
|-----------------|-------------|-------------|
| Label | Test Sample | DNA Control |
| Nuclei events | 18999.00 | 19344.00 |
| Singlet events | 18480.00 | 18883.00 |
| % sub G0/G1 | 0.88 | 0.61 |
| % G0/G1 | 60.05 | 51.11 |
| % S | 20.70 | 26.51 |
| % G2/M | 13.54 | 18.95 |
| % >4N | 1.26 | 0.84 |

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| No. | Element |
|-----|--|
| 1 | BD banner. Lists the BD logo, assay name, software version number, and report type. |
| 2 | Report header. Lists the cytometer name and serial number, software name and version number, operator name, and date and time the report was generated. |
| 3 | Acquisition information. Displays the tube name, sample ID, and acquisition date and time. |
| 4 | Acquisition plots. Displays the plots with gates. |
| 5 | Results summary table. Shows the number of events acquired and population percentages for each gate. |
| 6 | Report footer. Displays the regulatory status of the software and the page number of the report. |

More information

- [About the BD Cycletest assay \(page 56\)](#)

Troubleshooting information

See the troubleshooting sections in the *BD FACSVersé System User's Guide* and the *BD FACSVersé System Reference*.

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7

BD FastImmune assays

This chapter covers the following topics:

- [About the BD FastImmune assays \(page 70\)](#)
- [BD FastImmune assays workflow \(page 71\)](#)
- [Setting up the software \(page 71\)](#)
- [Running the samples \(page 74\)](#)
- [Viewing a BD FastImmune assay lab report \(page 77\)](#)

About the BD FastImmune assays

Introduction This topic provides a brief description of the BD FastImmune assays.

Purpose of the assays The BD FastImmune CD4 Intracellular detection assays are used to detect intracellular cytokines and the activation marker CD69 in antigen-activated CD4⁺ T lymphocytes in whole blood.

The following table shows the BD FastImmune assays supported by BD FACSuite software and the assays kit catalog numbers used in each assay.

| Assay name | Catalog No. | Reagent composition |
|----------------------|--|---|
| BD FastImmune CD4 3C | <ul style="list-style-type: none"> ● 340970 ● 340971 ● 340972 | <ul style="list-style-type: none"> ● Anti-Hu-IFN γ/CD69/CD4 ● Anti-Hu-IL-2/CD69/CD4 ● Anti-Hu-TNF α/CD69/CD4 |
| BD FastImmune CD4 4C | <ul style="list-style-type: none"> ● 337185 ● 337187 ● 337189 | <ul style="list-style-type: none"> ● Anti-Hu-IFN γ/CD69/CD4/CD3 ● Anti-Hu-IL-2/CD69/CD4/CD3 ● Anti-Hu-TNF α/CD69/CD4/CD3 |

Additional information

See the following technical data sheets:

- *BD FastImmune 3-Color CD4 Intracellular Cytokine Detection Kit*
- *BD FastImmune 4-Color CD4 Intracellular Cytokine Detection Kit*

BD FastImmune assays workflow

Introduction This topic guides you through a typical workflow when running samples stained with either the 3-color or 4-color BD FastImmune intracellular cytokine detection kit. See [About the BD FastImmune assays \(page 70\)](#) for a list of supported assay kits.

Workflow The stages in this workflow include:

| Stage | Description |
|-------|--|
| 1 | Setting up the software (page 71) |
| 2 | Running the samples (page 74) |
| 3 | Viewing a BD FastImmune assay lab report (page 77) |

Setting up the software

Introduction This topic describes how to set up the software to run one of the BD FastImmune assays.

- Before you begin**
1. Prepare and stain the samples according to the instructions in the technical data sheet.
 2. Verify that Performance QC was run and that it passed.
 3. Run Assay and Tube Settings Setup for the BD FastImmune assays and verify that status is **OK**.

See topics on running assay and tube settings setup in the *BD FACSVerser System User's Guide*.

Procedure

To set up the software:

1. From the Home page, click the **Acquire Data in Worklist** shortcut.

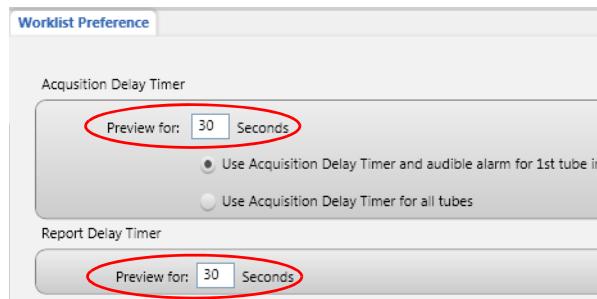


A new worklist opens.

2. From the menu bar, select **Tools > Preferences**.
3. In the **Worklist Preference** tab, set the preview time for the:
 - Acquisition delay timer
 - Report delay timer

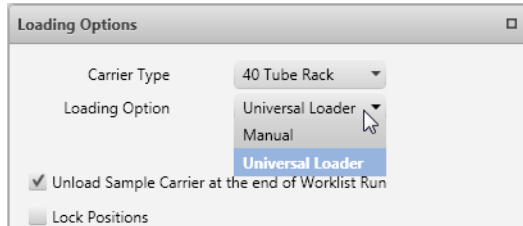
Ensure that the preview time is adequate to review the data and gates before acquisition begins.

Ensure that you have sufficient sample to accommodate increased preview times.

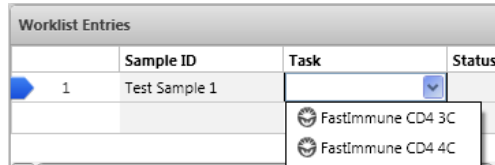


4. Set other worklist preferences as necessary. See topics about setting worklist preferences in the *BD FACSVerser System Reference* for more information.

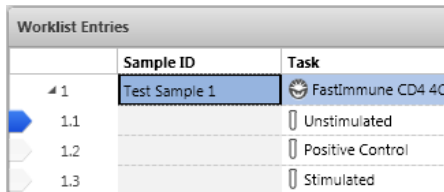
5. Select loading options in the **Loading Options** panel.



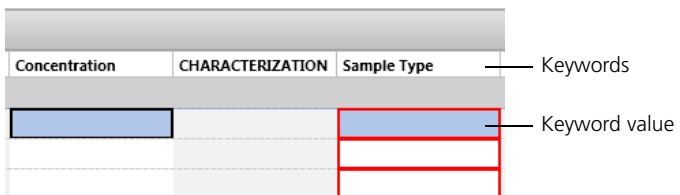
6. In the worklist, type a sample ID for the first entry and select one of the BD FastImmune assays from the **Task** menu.



All tubes associated with the assay are added to the entry. This example shows the tubes added for the BD FastImmune CD4 4C assay.



7. Scroll to the right to see system keywords. Text boxes outlined in red indicate that a keyword value is required.



8. Type in text for the following keywords:

- Required
 - Antigen
 - Cytokine
 - Sample type
- Optional
 - Concentration
 - Incubation time

If you do not enter values for the required keywords, the worklist status will remain **Not Ready** and you will be unable to run the worklist.

9. Repeat [step 6](#) through [step 8](#) for the remaining samples.

Next step

[Running the samples \(page 74\)](#)

Running the samples

Introduction

This topic describes how to run samples stained with one of the BD FastImmune intracellular cytokine detection kits.

Before you begin

Set up the software. See [Setting up the software \(page 71\)](#).

Procedure

To run the samples:

1. In the worklist, make sure that the **Status** column displays *Ready* for all entries and *Ready to Acquire* for all tubes.

| Worklist Entries | | | |
|------------------|---------------|-------------------|------------------|
| | Sample ID | Task | Status |
| 1 | Test Sample 1 | FastImmune CD4 4C | Ready |
| 1.1 | | Unstimulated | Ready To Acquire |
| 1.2 | | Positive Control | Ready To Acquire |
| 1.3 | | Stimulated | Ready To Acquire |

2. Click **Run All** in the **Worklist Controls** bar.



3. (Optional) In the dialog, save the worklist with a unique name.
4. Load the first Unstimulated sample on the cytometer using the loading method you selected in [step 5 of Setting up the software \(page 71\)](#).

Events are displayed in the plots in the acquisition worksheet.

5. (Optional) Click **Stop Timer** in the **Worklist Controls** bar.

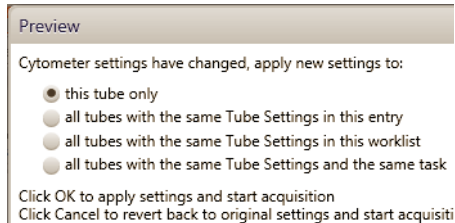
This stops the acquisition delay timer, but sample is still being aspirated into the cytometer.

6. If necessary, adjust FSC and SSC voltages to bring the lymphocytes within the Lymphocytes gate.

Alternatively, modify the Lymphocytes gate to enclose the lymphocytes.

7. Once settings are adjusted, click **Resume** to start acquisition.

The following dialog opens.



8. Select the option that you want to apply and then click **OK**.

The lab report is displayed after all tubes of the first entry have been acquired.

9. (Optional) Click **Stop Timer** to view the lab report plots and make adjustments to the gates, if necessary.
10. Click **Resume** to continue to the next entry.

Next step

[Viewing a BD FastImmune assay lab report](#)

Additional information

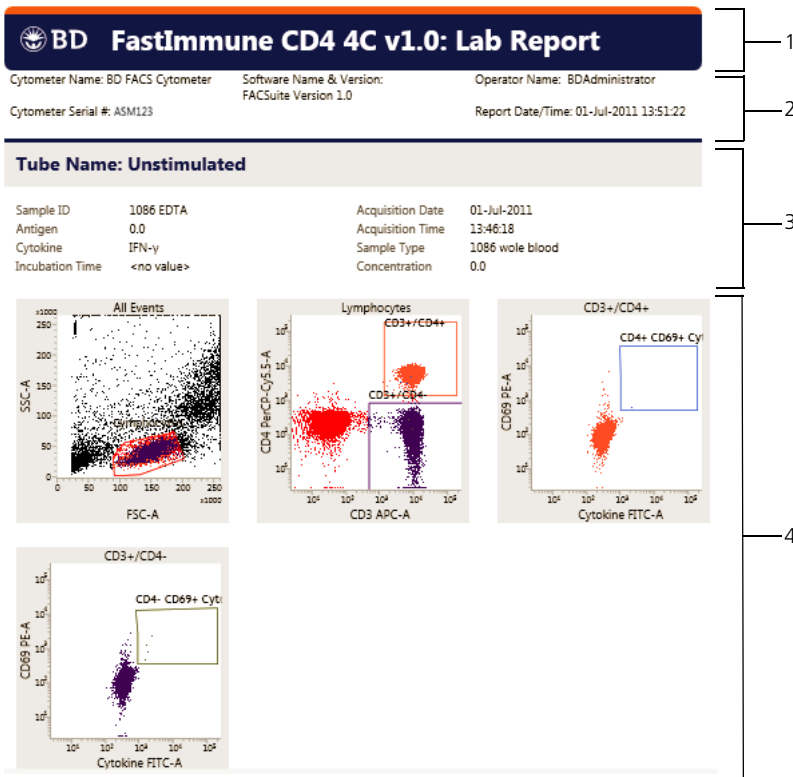
In the *BD FACSVerser System User's Guide*, see topics on running assay and tube settings setup and building a new worklist.

Viewing a BD FastImmune assay lab report

Introduction

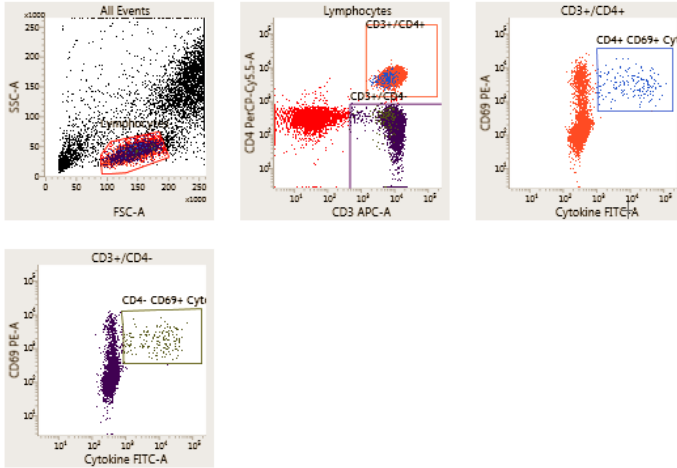
This topic shows an example lab report for the BD FastImmune assay and describes the elements in the report.

Example lab report



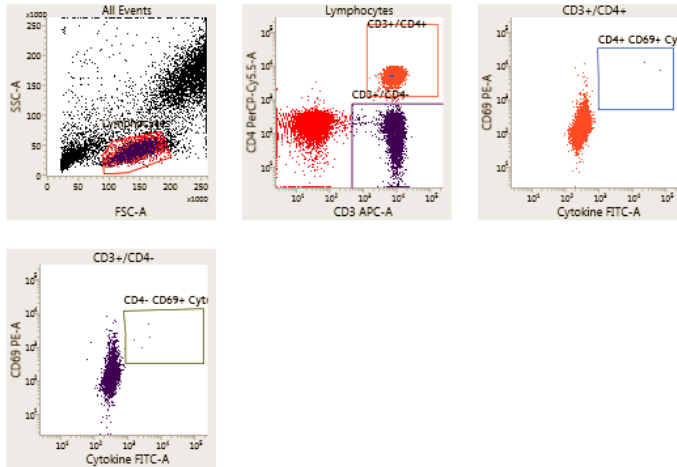
Tube Name: Positive Control

| | | | |
|-----------------|---------------|------------------|------------------|
| Sample ID | 1086 EDTA | Acquisition Date | 01-Jul-2011 |
| Antigen | SEB | Acquisition Time | 13:51:45 |
| Cytokine | IFN- γ | Sample Type | 1086 whole blood |
| Incubation Time | <no value> | Concentration | 2.0 |



Tube Name: Stimulated

| | | | |
|-----------------|---------------|------------------|------------------|
| Sample ID | 1086 EDTA | Acquisition Date | 01-Jul-2011 |
| Antigen | LPS | Acquisition Time | 13:57:22 |
| Cytokine | IFN- γ | Sample Type | 1086 whole blood |
| Incubation Time | <no value> | Concentration | 1.0 |



| Result Summary | | | |
|---------------------------------------|--------------|------------------|------------|
| | Unstimulated | Positive Control | Stimulated |
| Lymphocyte # Events | 46327.0 | 38555.0 | 40053.0 |
| CD3+ CD4+ Lymphocyte # Events | 17607.0 | 14594.0 | 14804.0 |
| CD4+ CD69+ Cytokine # Events | 3.0 | 281.0 | 6.0 |
| FITC MFI of CD3+ CD4+ CD69+ Cytokine+ | 1695.0 | 21351.6 | 44077.2 |
| Cytokine+ % of CD4+ Lymphocytes | 0.02 | 1.93 | 0.04 |
| CD3+ CD4- Lymphocyte # Events | 12378.0 | 10796.0 | 10912.0 |
| CD4- CD69+ Cytokine # Events | 5.0 | 304.0 | 8.0 |
| FITC MFI of CD3+ CD4- CD69+ Cytokine+ | 2677.4 | 15332.7 | 2432.5 |
| Cytokine+ % of CD4- Lymphocytes | 0.04 | 2.82 | 0.07 |

| Cross Tube Results | |
|--------------------------------|---------|
| Label | Results |
| Cell Response CD4+ Lymphocytes | 0.02 |
| Cell Response CD4- Lymphocytes | 0.03 |

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| No. | Element |
|-----|--|
| 1 | BD banner. Lists the BD logo, assay name, software version number, and report type. |
| 2 | Report header. Lists the cytometer name and serial number, software name and version number, operator name, and date and time the report was generated. |
| 3 | Acquisition information. Displays the tube name, sample ID, and acquisition date and time. |
| 4 | Acquisition plots. Displays the plots with gates. |
| 5 | Results summary table. Shows the number of events acquired and population percentages for each gate. |
| 6 | Cross tube results. Displays the CD4 ⁺ and CD4 ⁻ cell response. |
| 7 | Report footer. Displays the regulatory status of the software and the page number of the report. |

More information

- [About the BD FastImmune assays \(page 70\)](#)

Troubleshooting information

See the troubleshooting sections in the *BD FACSVerser System User's Guide* and the *BD FACSVerser System Reference*.

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