BD OneFlow™ Application Guide for Plasma Cell Disorders

For BD OneFlow™ PCST and BD OneFlow™ PCD

IVD CE

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Becton, Dickinson and Company
BD Biosciences
2350 Qume Drive
San Jose, CA 95131 USA

EC REP **Benex Limited**
Pottery Road, Dun Laoghaire
Co. Dublin, Ireland
Tel +353.1.202.5222
Fax +353.1.202.5388

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

Australian and New Zealand Distributors:

Becton Dickinson Pty Ltd.
66 Waterloo Rd
Macquarie Park NSW 2113
Australia

Becton Dickinson Ltd.
14b George Bourke Drive
Mt Wellington, Auckland, 1060
New Zealand

bdbiosciences.com
ClinicalApplications@bd.com

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Regulatory information

The BD FACSCanto II flow cytometer is a Class 1 laser product.

For In Vitro Diagnostic Use.

History

Revision	Date	Change made
23-16887-00	12/2015	Initial release
23-16887-01	11/2019	Removed CD from the installer description. Removed the cell range. Updated Australian and New Zealand addresses. Updated the BD Logo.

Contents

Chapter 1: Overview **5**

Overview of the BD OneFlow system 6

Workflows for BD OneFlow PCST and BD OneFlow PCD 7

Chapter 2: Sample preparation **9**

Washing the specimen 10

Staining the specimen 11

Chapter 3: Sample acquisition **15**

Setting up the experiment 16

Acquiring the stained sample 22

Chapter 4: Data analysis **27**

Analyzing the data using BD FACSDiva software 28

Chapter 5: Troubleshooting **37**

1 Overview

This chapter covers the following topics:

- Overview of the BD OneFlow system (page 6)
- Workflows for BD OneFlow PCST and BD OneFlow PCD (page 7)

Overview of the BD OneFlow system

About the system

The BD OneFlow™ system provides a comprehensive set of reagents and protocols to reproducibly set up the flow cytometer and stain patient specimens. The consistent instrument setup and sample staining enable you to acquire and analyze patient specimens for immunophenotyping of normal and aberrant cell populations in a manner compatible with that prescribed by the EuroFlow™ Consortium.

The BD OneFlow™ PCST and BD OneFlow™ PCD tubes are used to stain patient bone marrow specimens. The stained samples are acquired on the cytometer and then analyzed to identify normal and aberrant plasma cells.

Materials needed

- BD OneFlow™ PCST
  - Catalog No. 659912
- BD OneFlow™ PCD
  - Catalog No. 659913
- BD FACSDiva™ CS&T IVD beads (CS&T IVD beads)
  - Catalog No. 656046 or 656047
- BD OneFlow™ Setup Beads
  - Catalog No. 658620
- BD® FC Beads 8-color kit for BD OneFlow™ Assays (BD FC beads)
  - Catalog No. 658621
- Templates installer for BD OneFlow Assays
  - Catalog No. 659305
- BD FACSCanto™ II flow cytometer with a 3-laser, 8-color, 4-2H-2V BD default optical configuration, running BD FACSDiva™ software v8.0.1 or later
- FIX & PERM® Cell Fixation & Cell Permeabilization kit

Workflows for BD OneFlow PCST and BD OneFlow PCD

Specimen preparation

Task	Reagents or materials	Template needed	Outcome
Washing the specimen	Patient specimen Wash buffer	None	Washed patient specimen is ready for staining.
Staining the specimen	BD OneFlow PCST or BD OneFlow PCD FIX & PERM Wash buffer	None	Stained patient specimen is ready for acquisition.

Sample acquisition

Task	Reagents or materials	Template needed	Outcome
Import the appropriate OneFlow template.	None	OneFlow PCST or OneFlow PCD	The appropriate OneFlow template is imported into an experiment, and application settings are applied.
Acquiring the stained sample	Stained patient sample	The appropriate BD OneFlow Acquisition worksheet	The FCS file is generated.

Data analysis

Task	Reagents or materials	Template needed	Outcome
Analyzing the data using BD FACSDiva software	FCS file for patient sample	The appropriate BD OneFlow Analysis worksheet	Patient plasma cell populations are identified.

2

Sample preparation

This chapter covers the following topics:

- Washing the specimen (page 10)
- Staining the specimen (page 11)

Washing the specimen

About the specimens

This procedure works for bone marrow specimens.

It is crucial that all of the specimens stained using BD OneFlow PCST and BD OneFlow PCD are treated in the same manner. BD OneFlow PCST contains antibodies which recognize Igκ and Igλ found in the cytoplasm of plasma cells. Therefore, to avoid interference from serum antibodies found in the specimen, you must prewash the specimen three times before you stain it using BD OneFlow PCST and BD OneFlow PCD.

Preparing the specimen

1. For each specimen, label a 15-mL conical tube with the specimen ID.
2. Invert the specimen in the collection tube 10 times to mix well.
3. Add 300 µL of the patient specimen to the labeled conical tube.
4. Add 10 mL of wash buffer (filtered PBS + 0.5% BSA + 0.1% sodium azide).
5. Invert the tube 3–5 times to mix well.
6. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.
7. Remove the supernatant without disturbing the cell pellet.
8. Vortex the tube until no cell aggregates remain before adding wash buffer.

   Note: It is important to completely resuspend the cell pellet between each wash.
9. Repeat steps 4–8 twice for a total of three washes.
10. Resuspend the cell pellet in 200 µL of wash buffer to give a final volume of approximately 300 µL.
11. Vortex vigorously 3–5 seconds to completely resuspend the cell pellet.

Note: Start staining the specimen using the BD OneFlow PCST or BD OneFlow PCD tube within 30 minutes of the last wash. Store the washed specimen at 20°C–25°C until you stain it.

Staining the specimen

About the tubes

The BD OneFlow PCST and BD OneFlow PCD reagents are very sensitive to moisture. Ensure the pouch is completely resealed after removing a tube. Do not remove the desiccant from the reagent pouch.

Write the current date on the pouch label when it is first opened. Use the tubes from that pouch within one month before opening the next one.

About fixing and permeabilizing the cells

It is crucial that all of the specimens stained using BD OneFlow PCST and BD OneFlow PCD are treated in the same manner. In particular, make sure that you treat the PCD-stained specimens with FIX & PERM, as described in the protocol. This will ensure that the scatter properties of the cells will be the same for both of the tubes.

The volumes for the cell fixation and permeabilization steps are important. After using FIX & PERM Reagent A to fix the cells, wash them, centrifuge them, and remove the supernatant. We recommend that you measure the residual volume and then add wash buffer to give a final volume of 100 µL prior to adding FIX & PERM Reagent B. This will ensure the cells are completely permeabilized using FIX & PERM Reagent B.

Staining the specimen

1. Make sure that the pouches are at 20°C–25°C before opening them.

2. For each patient specimen, remove a BD OneFlow PCST (S) or BD OneFlow PCD tube from its pouch. Do not remove the BD OneFlow PCST (C) tube from its pouch at this time.
3. Place the tubes in a rack, protected from light.
4. Immediately reseal the pouch with any unused tubes.
5. Write the patient ID on the appropriate tube label within the area provided.
6. Vortex washed specimen 3–5 seconds to mix well.
7. Add 50 µL of wash buffer (filtered PBS + 0.5% BSA + 0.1% sodium azide) and 50 µL of washed patient specimen to the tube. Vortex vigorously 3–5 seconds to mix well.
8. Incubate for 30 minutes at 20°C–25°C in the dark.
9. Add 1.5 mL of wash buffer to each tube. Vortex vigorously 3–5 seconds to mix well.
10. Add an additional 1.5 mL of wash buffer. Vortex gently to mix.
11. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.
12. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 µL of residual liquid in the tube.
13. Vortex vigorously until the cell pellet is completely resuspended.
14. Add 100 µL of FIX & PERM Reagent A (fixation solution) to the tube. Vortex vigorously 3–5 seconds to mix well.
15. Incubate for 15 minutes at 20°C–25°C in the dark.
16. Add 1.5 mL of wash buffer. Vortex vigorously 3–5 seconds to mix well.
17. Add an additional 1.5 mL of wash buffer. Vortex gently to mix.
18. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.

19. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 µL of residual liquid in the tube.
20. Vortex vigorously until the cell pellet is completely resuspended.

    Note: If you are unable to obtain a single-cell suspension, see Troubleshooting.
21. Measure the volume in each tube using a pipet and add wash buffer to give a final volume of 100 µL in each tube. Vortex 3–5 seconds to mix well.

    Note: It is important to have a final volume of 100 µL in each tube so that all of the cells will be completely permeabilized in steps 24–27. If you are staining specimens using BD OneFlow PCST and BD OneFlow PCD, set aside the BD OneFlow PCD tube until step 24.
22. Remove the appropriate number of BD OneFlow PCST (C) tubes from the pouch and reseal the pouch immediately.

    Note: Write the current date on the pouch label when it is first opened. Use the tubes from that pouch within one month before opening the next one.
23. Write the patient ID on the BD OneFlow PCST (C) tube label within the area provided.
24. Add 100 µL of FIX & PERM Reagent B (permeabilization solution) to the BD OneFlow PCST (C) tube and the BD OneFlow PCD tube. Vortex the BD OneFlow PCD tube vigorously 3–5 seconds to mix well.
25. Transfer 100 µL of the sample from the BD OneFlow PCST (S) tube to the corresponding BD OneFlow PCST (C) tube.

    Note: Make sure that the patient ID numbers on the two tubes are the same.
26. Vortex the BD OneFlow PCST (C) tube vigorously 3–5 seconds to mix well.
27. Incubate both tubes for 15 minutes at 20°C–25°C in the dark.

28. Add 1.5 mL of wash buffer. Vortex vigorously 3–5 seconds to mix well.
29. Add an additional 1.5 mL of wash buffer. Vortex gently to mix.
30. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.
31. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 µL of residual liquid in the tube.
32. Add 200 µL of wash buffer to each tube. Vortex vigorously 3–5 seconds to completely resuspend the cell pellet.

Next step

Acquire the stained sample within 1 hour of staining. Store the stained sample at 2°C–8°C in the dark until acquisition.

3

Sample acquisition

This chapter covers the following topics:

- Setting up the experiment (page 16)
- Acquiring the stained sample (page 22)

Setting up the experiment

About linking and unlinking compensation

When you create a new experiment, you must apply the correct application settings. Before applying the correct application settings, you first link the appropriate compensation matrix to the experiment and then unlink the compensation matrix. Unlinking the compensation matrix allows updated application settings to be applied, thus giving photomultiplier tube voltages (PMTVs) that will result in correct target median fluorescence intensity (MFI) values, while retaining compensation values. When you apply the application settings you keep the compensation values.

Before you begin

1. In BD FACSDiva software v.8.0.1 or later, ensure that cytometer warmup is complete, fluidics startup has been performed, and that the cytometer is in the default 4-2H-2V configuration.
2. Verify that the daily performance check was completed and passed for the default 4-2H-2V configuration using CS&T IVD beads within the past 24 hours. See the *Instrument Setup Guide for BD OneFlow™ Assays*.
3. Recommended: confirm that the PMTVs are still within their daily target ranges. See the chapter for daily setup in the *Instrument Setup Guide for BD OneFlow™ Assays*.
4. Make sure that you have installed the OneFlow PCST and OneFlow PCD templates. See the *Instrument Setup Guide for BD OneFlow™ Assays* or the Instructions for Use for the appropriate BD OneFlow multicolor tube.

Setting up the experiment

1. Create a new experiment.
   a. From the menu bar, select **Experiment > New Experiment > Blank Experiment**. Click **OK**.

      Note: You can also create an experiment directly from the **Browser** using the **Experiment** icon.

b. If prompted by the **CST Mismatch** dialog, select **Use CST Settings**.

c. Rename the experiment according to your laboratory practice.

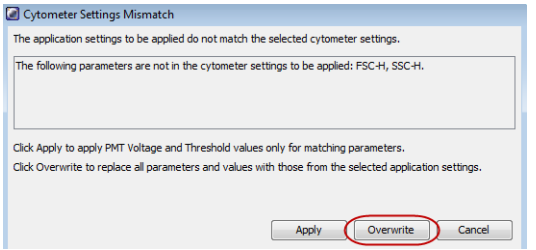
2. Link compensation.

a. In the **Browser**, right-click **Cytometer Settings.**

b. From the menu, select **Link Setup**.

c. Select the appropriate compensation matrix calculated using BD FC beads within the past 31 days. Click **Link**.

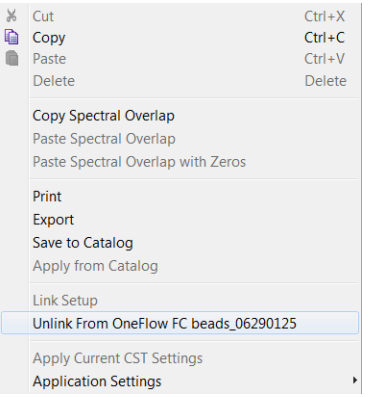
d. If prompted by the **Cytometer Settings Mismatch** dialog, select **Overwrite**.



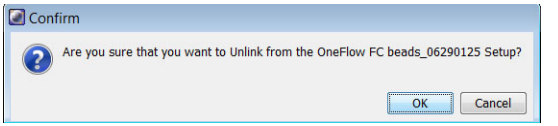
3. Unlink compensation.

a. In the **Browser**, right-click **Cytometer Settings.**

b. From the menu, select **Unlink From** and select the previously linked compensation setup.



c. From the **Confirm** dialog that opens, click **OK** to unlink from the previously linked compensation setup.



4. Apply application settings.

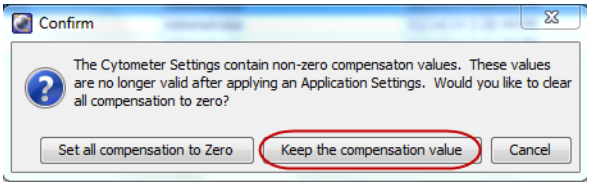
   a. In the **Browser**, right-click **Cytometer Settings**.

   b. From the menu, select **Application Settings** > **Apply**.

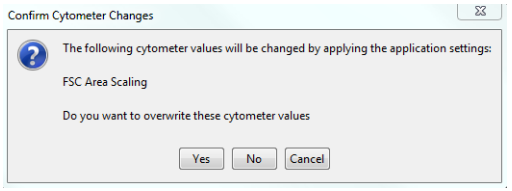
   c. Select the most recent application settings. Click **Apply**.

   Note: Confirm that the most recent application settings were created within the past 31 days using the BD OneFlow Setup beads. The application settings are created in the monthly setup as described in the *Instrument Setup Guide for BD OneFlow™ Assays*.

d. When prompted by the **Confirm** dialog, select **Keep the compensation value**.



e. If prompted by the **Confirm Cytometer Changes** dialog, click **Yes** to overwrite the cytometer values for **FSC Area Scaling**.



5. Import the appropriate OneFlow template.

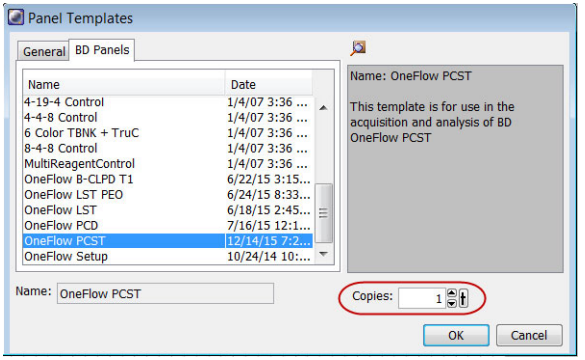
   a. Select the experiment in the **Browser** and then select **Experiment > New Specimen** from the menu bar**.**

      The **Panel Templates** dialog opens.

   b. Navigate to the **BD Panels** tab and select the appropriate OneFlow template.

      Note: Make sure that you select the template for the BD OneFlow tube that you are acquiring.

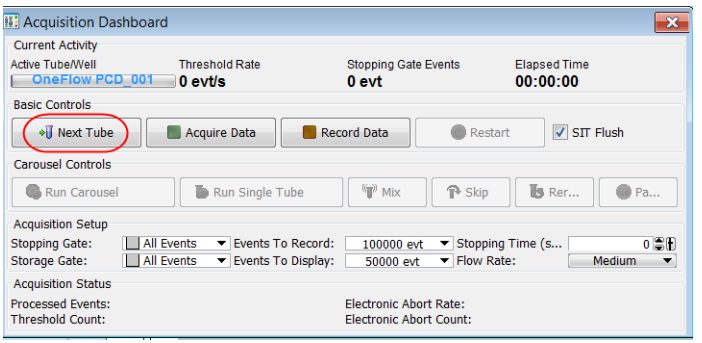
c. Indicate the number of patient specimens you want to acquire using the **Copies** field.



d. Click **OK**.

e. Rename each specimen, for example, with the appropriate patient ID in front of the specimen name.

Note: If you have to re-run a particular patient specimen, set the current tube pointer to the tube you wish to re-run. Click **Next Tube** in the **Acquisition Dashboard** to create another tube for that patient. Do not use the new tube icon to create the additional tube to be acquired because the labels and barcode fields will not be populated.



6. Confirm that all of the voltages are the same as those set as application settings.

a. In the **Browser**, select the application settings that you want to confirm.

b. In the **Inspector**, navigate to the **Parameters** tab to view the voltages in the application settings.

c. From the menu bar, select **Cytometer** > **Catalogs**.

   The **Catalogs** dialog opens.

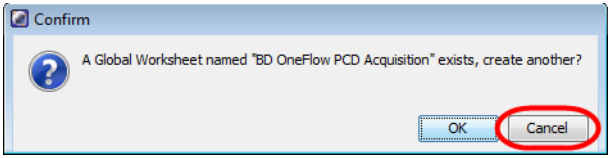
d. Navigate to the **Application Settings** tab.

e. Select the application settings used in the current experiment. Click **View**.

f. Confirm that the voltages in the catalog are the same as those in the application settings.

g. Click **Close** in the **Catalogs** dialog.

Note: If you want to acquire additional patient samples in the experiment, repeat step 5 to add new specimens. Two **Confirm** dialogs will open asking if you want to create another Acquisition worksheet or another Analysis worksheet. Click **Cancel** in each dialog.

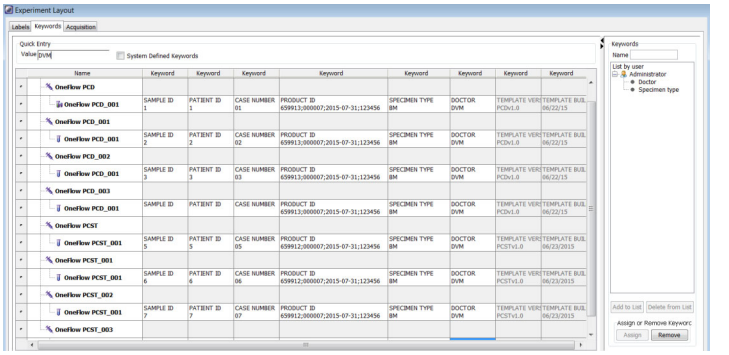


7. Scan the barcode on the tube label into the **Product ID** keyword field.

   a. From the menu bar, select **Experiment** > **Experiment Layout** and navigate to the **Keywords** tab.

   b. Highlight the **Product ID** keyword for the appropriate tube, and scan the barcode on the appropriate BD OneFlow tube label.

Note: If you cannot scan the barcode on the tube label, see Troubleshooting.



c. Manually add the appropriate information to the remaining keywords, as needed.

d. Click **OK** to close the **Experiment Layout**.

Acquiring the stained sample

Acquiring the tube

1. Vortex the tube 3–5 seconds at low speed immediately before acquiring the tube on the cytometer.
2. In the **Browser**, expand the appropriate specimen and set the current tube pointer to that tube.
3. Install the stained tube on the cytometer. Adjust the flow rate to **Medium** in the **Acquisition Dashboard**. Click **Acquire Data**.
4. Verify that the population is on scale and adjust the gate in the first plot of the appropriate BD OneFlow Acquisition worksheet to exclude debris, if needed.
5. Click **Record Data** in the **Acquisition Dashboard** and collect 100,000 total events.

Note: The template automatically collects 100,000 total events. Use the menu in the **Acquisition Dashboard** to select a different number of events to acquire, if needed.

Inspecting the BD OneFlow PCST Acquisition worksheet

1. Inspect the dot plots on the PCST acquisition worksheet, and adjust the gates as needed.

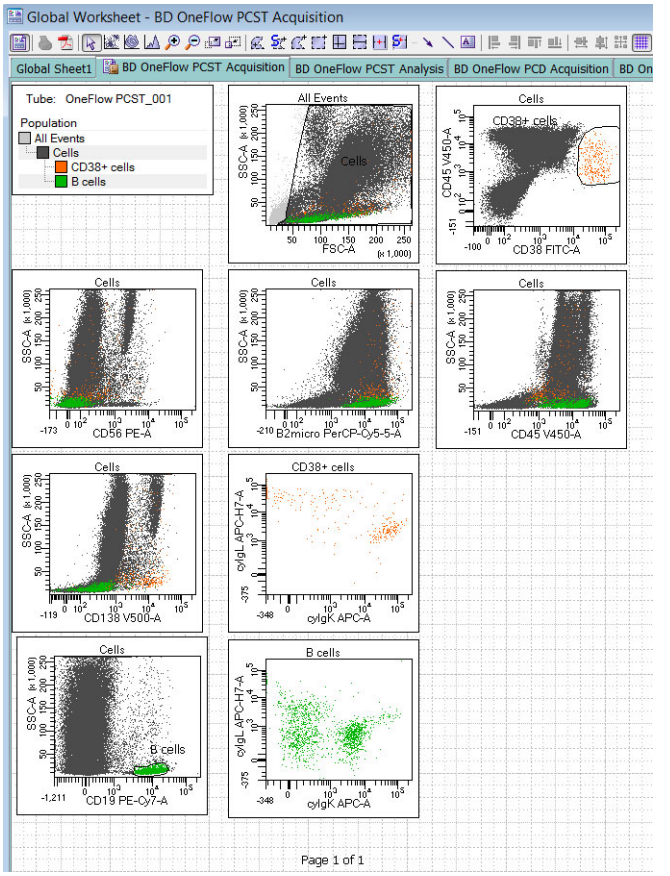
   The FSC-A vs SSC-A dot plot is used to identify cells.

   The CD38 FITC-A vs CD45 V450-A dot plot is used to identify $CD38^+$ cells.

   The CD19 PE-Cy7-A vs SSC-A dot plot is used to identify B cells.

   The dot plots for the remaining markers do not contain gates and are included to ensure that the antibodies can stain cells in the specimen, therefore serving as an internal quality control for the tube.

Examine the cyIgK APC-A vs cyIgL APC-H7-A dot plots to assess the clonality of the CD38$^+$ cells and the B cells.



2. Continue until all of the tubes have been acquired.
3. From the menu bar, select **File > Export > Experiments**, and select the **Directory Export** option. Click **OK**.

Inspecting the BD OneFlow PCD Acquisition worksheet

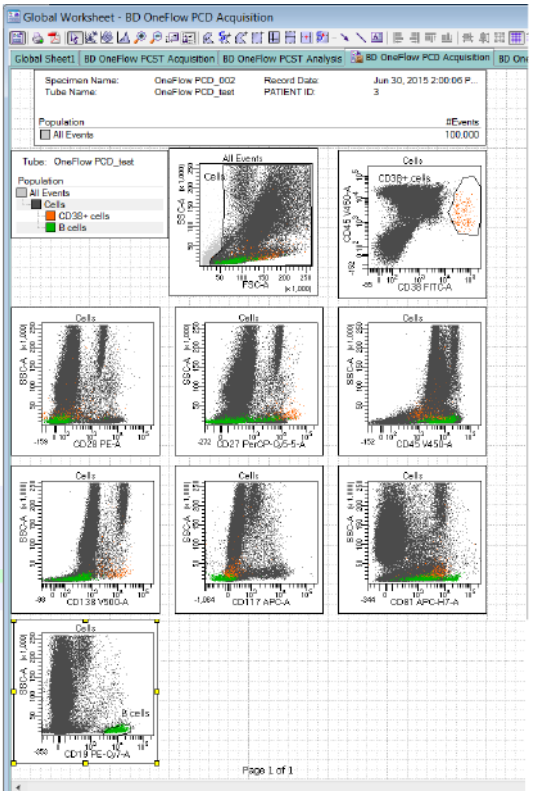
1. Inspect the dot plots on the PCD acquisition worksheet, and adjust the gates as needed.

   The FSC-A vs SSC-A dot plot is used to identify cells.

The CD38 FITC-A vs CD45 V450-A dot plot is used to identify $CD38^+$ cells.

The CD19 PE-Cy7-A vs SSC-A dot plot is used to identify B cells.

The dot plots for the remaining markers do not contain gates and are included to ensure that the antibodies can stain cells in the specimen, therefore serving as an internal quality control for the tube.



2. Continue until all of the tubes have been acquired.

3. From the menu bar, select **File > Export > Experiments**, and select the **Directory Export** option. Click **OK**.

4

Data analysis

This chapter covers the following topic:

- Analyzing the data using BD FACSDiva software (page 28)

Analyzing the data using BD FACSDiva software

About the dot plots

Some of the dot plots might look different from those in other experiments. The initial FSC-A vs SSC-A dot plot to identify cells and eliminate debris may appear compressed. This is a consequence of the target values used to create the application settings. The values are specified by the EuroFlow Consortium.

Analyzing BD OneFlow PCST

1. From the menu bar, select **File > Import > Experiments**.
2. Select the experiment that you want to analyze. Click **Import**.

   The experiment with the associated acquisition and analysis worksheets opens.
3. Select the **BD OneFlow PCST Analysis** worksheet tab.
4. Inspect the dot plots on page 1 of the PCST analysis worksheet, and adjust the gates as needed.

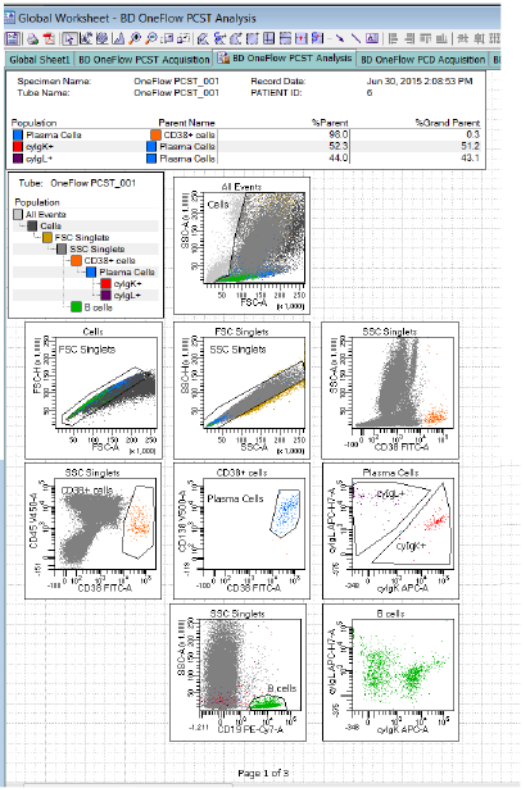
   Note: Enlarge the plot while adjusting the gates so you can more readily see the populations of interest.

   The first three dot plots on page 1 of the analysis worksheet identify the FSC and SSC singlets. Debris and doublets are excluded by adjusting the gates.

   The $CD38^{+}$ cells are identified in the CD38 FITC-A vs CD45 V450-A dot plot, and then plasma cells are identified in the CD38 FITC-A vs CD138 V500-A dot plot. The plasma cells are subsequently characterized by gating on the cells expressing cyIgκ and cyIgλ. These three dot plots are repeated at the top of page 2 of the PCST analysis worksheet for reference. The CD38 FITC-A vs SSC-A dot plot is included for informational purposes to allow for the visualization of $CD38^{bright}$ cells.

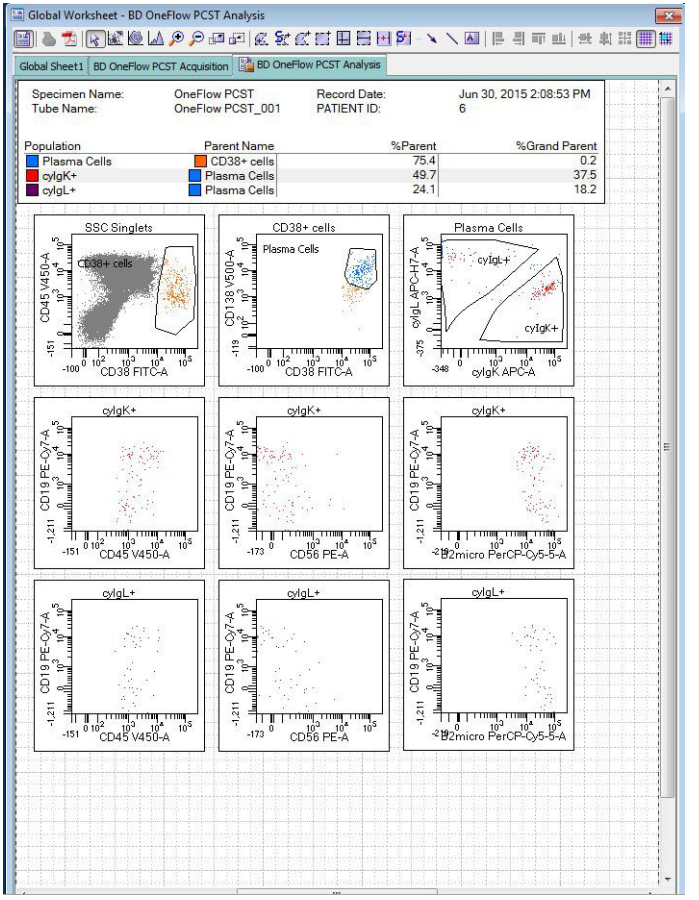
   B cells are identified in the CD19 PE-Cy7-A vs SSC-A dot plot and then characterized in the cyIgK APC-A vs cyIgL APC-H7-A dot plot.

Note: These are examples of normal bone marrow. Patient samples may look different.



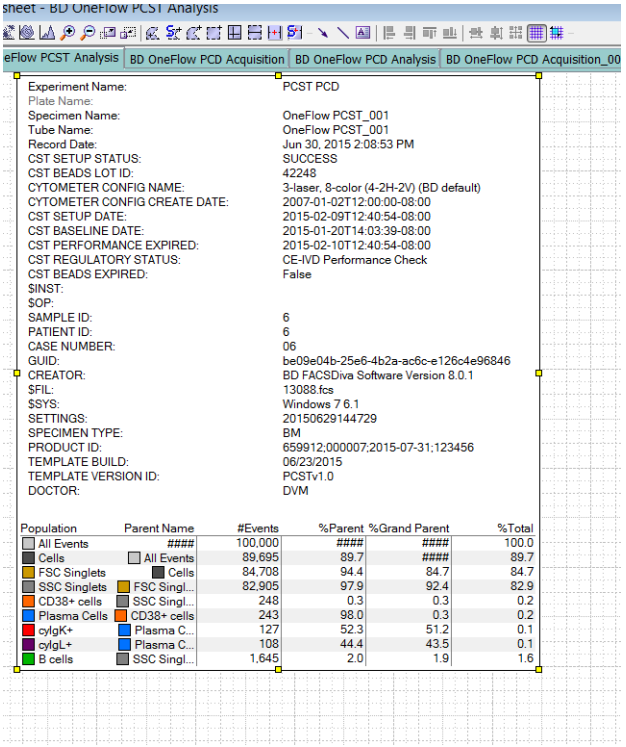
5. Inspect the dot plots on page 2 of the PCST analysis worksheet.

The cyIgκ$^+$ plasma cells and cyIgλ$^+$ plasma cells are further characterized according to the levels of CD19, CD45, CD56, and β2-Microglobulin expression.



6. Examine the results in the statistics box on page 3 of the PCST analysis worksheet.

Confirm that all of the keywords are present in the statistics box. If any of the keywords are missing, see Troubleshooting.



7. Perform other analyses as needed.

   Note: The plots shown in the figures of the PCST analysis worksheet are for normal populations of cells from bone marrow samples. If your analysis shows cell populations which fall outside of the provided gates, they might represent aberrant cell populations, and will require further analysis.

8. Save the PCST analysis worksheet as a PDF.

   Note: The PCST analysis worksheet is a global worksheet. Any gates that are adjusted when analyzing a sample on a global worksheet will be changed in previously analyzed files. Previously saved PDFs will not change, but if you go back to a

previously analyzed global worksheet, you will have to readjust the gates so they match what they were before.

9. Optional: click **Print** to print the PCST analysis worksheet.
10. Analyze the next sample.

Analyzing BD OneFlow PCD

1. From the menu bar, select **File > Import > Experiments**.
2. Select the experiment that you want to analyze. Click **Import**.

   The experiment with the associated acquisition and analysis worksheets opens.
3. Select the **BD OneFlow PCD Analysis** worksheet tab.
4. Inspect the plots on page 1 of the PCD analysis worksheet, and adjust the gates as needed.

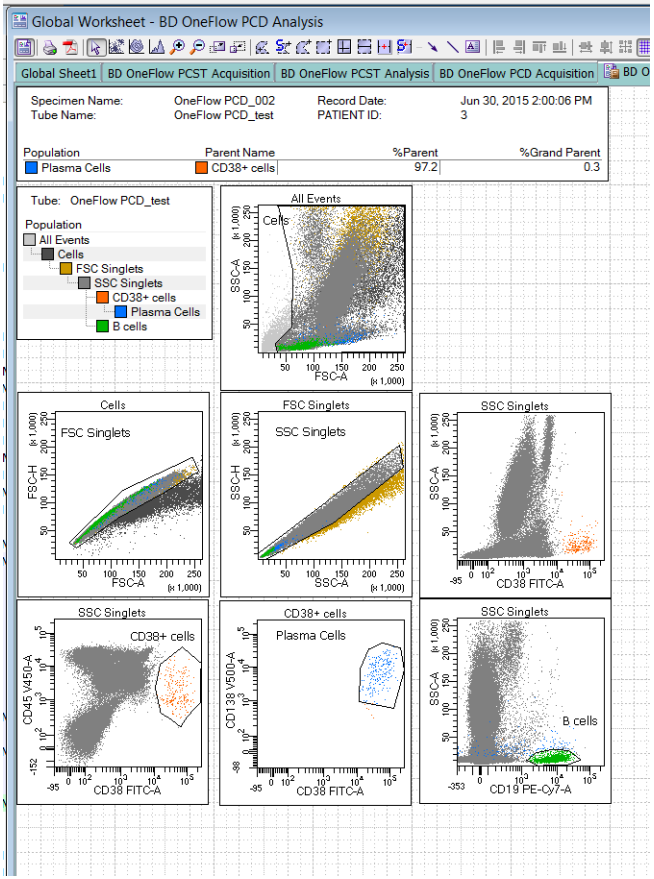
   Note: Enlarge the dot plot while adjusting the gates so you can more readily see the populations of interest.

   The first three dot plots on page 1 of the analysis worksheet identify the FSC and SSC singlets. Debris and doublets are excluded by adjusting the gates.

   The $CD38^{+}$ cells are identified in the CD38 FITC-A vs CD45 V450-A dot plot, and then plasma cells are identified in the CD38 FITC-A vs CD138 V500-A dot plot. These two dot plots are repeated at the top of page 2 of the PCD analysis worksheet for reference. The CD38 FITC-A vs SSC-A dot plot is included for informational purposes to allow for the visualization of $CD38^{bright}$ cells.

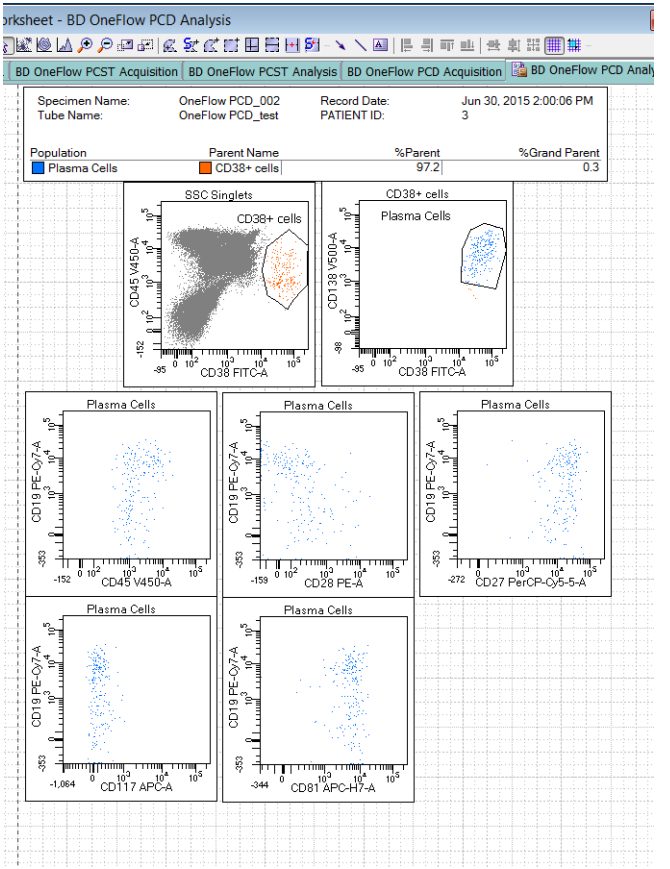
   B cells are identified in the CD19 PE-Cy7-A vs SSC-A dot plot.

Note: These are examples of normal bone marrow. Patient samples may look different.



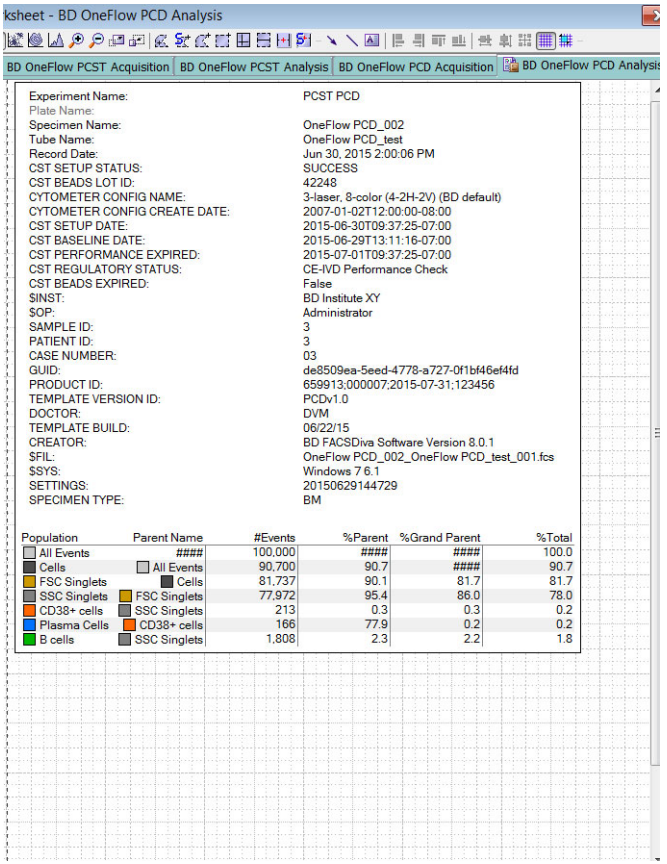
5. Inspect the dot plots on page 2 of the PCD analysis worksheet.

The dot plots on page 2 of the PCD analysis worksheet include markers that can help characterize the plasma cells as being normal or aberrant.



6. Examine the results in the statistics box on page 3 of the PCD analysis worksheet.

Confirm that all of the keywords are present in the statistics box. If any of the keywords are missing, see Troubleshooting.



7. Perform further analyses as needed.

   Note: The plots shown in the figures of the PCD analysis worksheet are for normal populations of cells. If your analysis shows cell populations which fall outside of the provided gates, they might represent aberrant cell populations, and will require further analysis.

8. Save the PCD analysis worksheet as a PDF.

Note: The PCD analysis worksheet is a global worksheet. Any gates that are adjusted when analyzing a sample on a global worksheet will be changed in previously analyzed files. Previously saved PDFs will not change, but if you go back to a previously analyzed global worksheet, you will have to readjust the gates so they match what they were before.

9. Optional: click **Print** to print the PCD analysis worksheet.
10. Analyze the next sample.

5

Troubleshooting

This chapter covers the following topics:

- Templates do not import correctly (page 38)
- Problems using BD OneFlow PCST or BD OneFlow PCD (page 39)

Templates do not import correctly

You may observe that templates do not import correctly. For example, there might not be dot plots in the global worksheet, the plots from the wrong worksheet appear when you import a panel template, or the imported panel template does not include tubes.

If you suspect that the templates did not import correctly:

1. Close the current experiment.
2. Create a new experiment.
3. Re-import the panel template.

Problems using BD OneFlow PCST or BD OneFlow PCD

Problem	Possible cause	Solution
The resolution between debris and cells is poor.	Specimen was poorly lysed.	Repeat staining; vortex tubes until no cell aggregates remain before adding FIX & PERM Reagent A.
	Specimen is of poor quality.	Check cell viability.
	Instrument settings are inappropriate.	Follow proper instrument setup procedures. Optimize instrument settings as required.
The cytoplasmic staining (Igκ and Igλ) is dim.	The cells were not completely permeabilized.	Repeat staining; carefully measure the specimen volumes in the cell fixation and permeabilization steps such that the ratio of fixed sample to FIX & PERM Reagent B is 1:1.
Cells clump after being fixed.	Cells were not completely resuspended before fixing them.	Vortex tubes until no cell aggregates remain before adding FIX & PERM Reagent A.
	Cells were not thoroughly washed after fixing them.	Incubate the tubes for 2 minutes in the dark in wash buffer after they have been fixed using FIX & PERM Reagent A.

Problem	Possible cause	Solution
Staining is dim or fading.	Cell concentration was too high at the staining step.	Check the cell concentration and adjust as needed.
	Washed specimen was not stained within 30 minutes of the last wash.	Repeat staining with a freshly prepared specimen.
	The BD OneFlow tube was exposed to light for too long.	Repeat staining with a new BD OneFlow tube.
	Cells were not acquired within 1 hour of staining.	Repeat staining with a fresh specimen and acquire promptly.
Few or no cells are recorded.	Cell concentration was too low.	Resuspend fresh specimen at a higher concentration. Repeat staining and acquisition.
	Cytometer is malfunctioning.	Troubleshoot the instrument. See the cytometer instructions for use for more information.
Some of the dot plots are dimmed.	FSC-H and SSC-H were not selected when the application settings were created.	Check that FSC-H and SSC-H are selected on the **Parameters** tab of the **Inspector**.

Problem	Possible cause	Solution
The barcode on the tube label cannot be scanned.	The barcode on the tube label has been compromised.	Scan the barcode on the BD OneFlow pouch label into the **Product ID** keyword field in the **Experiment Layout**. Next, manually enter a semicolon (;) followed by the six-digit tube-specific ID, found adjacent to the barcode on the tube label, after the last digit of the barcode.
Some of the keywords are missing from the statistics box in the analysis worksheet.	BD FACSDiva software did not import all of the keywords into the panel template.	1. Navigate to the analysis worksheet. 2. Right-click the statistics box and select **Edit Stats View**. 3. In the **Header** tab, select the **All** checkbox. 4. Click **OK**.
The statement, **For in vitro diagnostic use**, does not appear in the footer of the analysis worksheet when it is printed.	The paper margins in the printer settings were changed.	1. From the BD FACSDiva software menu bar, select **File > Page Setup**. 2. Ensure that all of the margins are set to 2.54 cm or 1 inch, depending on your default standards. 3. Click **OK**.

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