BD OneFlow[™] Application Guide for Plasma Cell Disorders

For BD OneFlow[™] PCST and BD OneFlow[™] PCD



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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.

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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 TM 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 TM Consortium.
	The BD OneFlow TM PCST and BD OneFlow TM 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 TM 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.

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Sample preparation

This chapter covers the following topics:

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

Washing the specimen

About the	This procedure works for bone marrow specimens.						
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 Igk and Ig λ 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 μ L of the patient specimen to the labeled conical tube.						
	 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 $540g$ for 5 minutes at $20^{\circ}C-25^{\circ}C$.						
	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 μ L of wash buffer to give a final volume of approximately 300 μ L.						
	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 μ L prior to adding FIX & PERM Reagent B. This will ensure the cells are completely permeabilized using FIX & PERM Reagent B.
Staining the specimen	 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 μ L of wash buffer (filtered PBS + 0.5% BSA + 0.1% sodium azide) and 50 μ L 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.
- 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 540g for 5 minutes at 20°C–25°C.
- 12. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 μ L 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 540g 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 μ L in each tube. Vortex 3–5 seconds to mix well.

Note: It is important to have a final volume of $100 \ \mu$ L 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.
- 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 540g for 5 minutes at 20°C–25°C.
- 31. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 μ L 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.

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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

nd 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 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.

- 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*.
- 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.

Cytometer Settings Mismatch
The application settings to be applied do not match the selected cytometer settings.
The following parameters are not in the cytometer settings to be applied: FSC-H, SSC-H.
Click Apply to apply PMT Voltage and Threshold values only for matching parameters. Click Overwrite to replace all parameters and values with those from the selected application settings.
Apply Overwrite Cancel

- 3. Unlink compensation.
 - a. In the Browser, right-click Cytometer Settings.

🐰 Cut Ctrl+X 🗋 Сору Ctrl+C Paste Ctrl+V Delete Delete Copy Spectral Overlap Paste Spectral Overlap Paste Spectral Overlap with Zeros Print Export Save to Catalog Apply from Catalog Link Setup Unlink From OneFlow FC beads_06290125 Apply Current CST Settings Application 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 OneFlowTM 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.

OneFlow PCD 7/16/15 12:1 OneFlow PCST 12/14/15 7:2 OneFlow Setup 10/24/14 10:	Name A19-4 Control 4-4-8 Control 4-4-8 Control 6 Color TBNK + TruC 8-4-8 Control MultiReagenControl OneFlow B-CLPD T1 OneFlow LST FEO OneFlow LST	Date 1/4/07 3:36 1/4/07 3:36 1/4/07 3:36 1/4/07 3:36 6/22/15 3:15 6/24/15 8:33 6/24/15 2:45	Name: OneFlow PCST This template is for use in the acquisition and analysis of BD OneFlow PCST
OneFlow PCST 12/14/15 7:2 OneFlow Setup 10/24/14 10:	OneFlow PCD	7/16/15 12:1	
	OneFlow PCST OneFlow Setup	12/14/15 7:2 10/24/14 10: [•]	-

- 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.

Acquisition Dasht	ooard		
Current Activity			
Active Tube/Well OneFlow PCD_0	Threshold Rate 0 evt/s	Stopping Gate Events 0 evt	Elapsed Time 00:00:00
Basic Controls			
◆ij Next Tube	Acquire Data	Record Data	Restart 🛛 🔽 SIT Flush
Carousel Controls			
Run Carousel	🕞 Run Single Tube	e 🕅 Mix 🕈 S	Skip 🖪 Rer 🖉 Pa
Acquisition Setup			
Stopping Gate:	All Events Events To Re All Events Events To Di	ecord: 100000 evt ▼ St isplay: 50000 evt ▼ Flo	opping Time (s 0 🕃 🛉 ow Rate: Medium 💌
Acquisition Status			
Processed Events: Threshold Count:		Electronic Abort Rate:	

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.

sk UR	DVM S	rstern Defined Key	words							Keywords Name
	Name	Keyword	Keyword	Keyword	Keyword	Keyword	Keyword	Keyword	Keyword	Eist by user
	- X OneFlow PCD								^	Octor Specimen type
	0neFlow PCD_001	SAMPLE ID	PATIENT ID	CASE NUMBER 01	PRODUCT ID 659913;000007;2015-07-31;123456	SPECIMEN TYPE BM	DOCTOR DVM	TEMPLATE VER: PCDv1.0	TEMPLATE BUIL 06/22/15	
	ConeFlow PCD_001									
	OneFlow PCD_001	SAMPLE ID 2	PATIENT ID 2	CASE NUMBER 02	PRODUCT ID 659913;000007;2015-07-31;123456	SPECIMEN TYPE BM	DOCTOR DVM	TEMPLATE VERS PCDv1.0	TEMPLATE BUIL 06/22/15	
	- % OneFlow PCD_002									
	J OneFlow PCD_001	SAMPLE ID 3	PATIENT ID	CASE NUMBER 03	PRODUCT ID 659913;000007;2015-07-31;123456	SPECIMEN TYPE BM	DOCTOR DVM	TEMPLATE VERS PCDv1.0	TEMPLATE BUIL 06/22/15	
	- X OneFlow PCD_003									
	UneFlow PCD_001	SAMPLE ID	PATIENT ID	CASE NUMBER	PRODUCT ID 659913;000007;2015-07-31;123456	SPECIMEN TYPE BM	DOCTOR DVM	TEMPLATE VER PCDv1.0	TEMPLATE BUIL 06/22/15	
	- MoneFlow PCST									
	J OneFlow PCST_001	SAMPLE ID 5	PATIENT ID 5	CASE NUMBER 05	PRODUCT ID 659912;000007;2015-07-31;123456	SPECIMEN TYPE BM	DOCTOR DVM	TEMPLATE VERS PCSTv1.0	TEMPLATE BUIL 06/23/2015	
	- X OneFlow PCST_001									
	J OneFlow PCST_001	SAMPLE ID 6	PATIENT ID 6	CASE NUMBER 06	PRODUCT ID 659912;000007;2015-07-31;123456	SPECIMEN TYPE BM	DOCTOR DVM	TEMPLATE VERS PCSTv1.0	TEMPLATE BUIL 06/23/2015	
	Conellow PCST_002									
	0neFlow PCST_001	SAMPLE ID 7	PATIENT ID	CASE NUMBER 07	PRODUCT ID 659912;000007;2015-07-31;123456	SPECIMEN TYPE BM	DOCTOR DVM	TEMPLATE VER	TEMPLATE BUIL	Add to List Delete from
	A OneFlow PCST 003									Assign or Remove Key

- 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.
 In the Browser, expand the a current tube pointer to that t Install the stained tube on the to Medium in the Acquisition 		In the Browser , expand the appropriate specimen and set the current tube pointer to that tube.
		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	ts Some of the dot plots might look different from those in or experiments. The initial FSC-A vs SSC-A dot plot to identi and eliminate debris may appear compressed. This is a consequence of the target values used to create the applicat settings. The values are specified by the EuroFlow Consort	
Analyzing	1.	From the menu bar, select File > Import > Experiments.
BD OneFlow PCST	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 cyIg κ and cyIg λ . 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 cyIg κ^+ plasma cells and cyIg λ^+ plasma cells are further characterized according to the levels of CD19, CD45, CD56, and $\beta 2$ -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.

Experiment Name: PCST PCD Plate Name: OneFlow PCST_001 Specimen Name: OneFlow PCST_001 Tube Name: OneFlow PCST_001 Record Date: Jun 30, 2015 2:08:53 PM CST BEADS LOT ID: 42248 CYTOMETER CONFIG CREATE DATE: 2007-01-027112:00:00-08:00 CST BEADS LOT ID: 42248 CYTOMETER CONFIG CREATE DATE: 2015-01-20112:40:54-08:00 CST BEALIN CATE: 2015-01-20112:40:54-08:00 CST BEALIN CONFIG CREATE DATE: 2015-01-20112:40:54-08:00 CST BEALIN CONFIG 6 QUD: be00904b-25e6-4b2a-ac6c-e1260:496846	w PCST Analysis	BD OneFlow PC	D Acquisition	BD OneFlow	PCD Analysis BD C	neFlow PCD	Acqui	sitio	۱_
Plate Name: OneFlow PCST_001 Tube Name: OneFlow PCST_001 Record Date: Jun 30, 2015 206.53 PM CST SETUP STATUS: SUCCESS CST BEADS LOT ID: 42248 CYTOMETER CONFIG CREATE DATE: 2015-02.09112.40.54-08.00 CST SETUP DATE: 2015-02.09112.40.54-08.00 CST BEADS LARDERANCE EXPIRED: 2015-02.09112.40.54-08.00 CST BEADS LEXPIRED: CST PERFORMANCE EXPIRED: CST BEADS EXPIRED: False SINST: SOP: SAMPLE ID: 6 CAST SETUP STATUS: CE-IVD Performance Check CST BEADS EXPIRED: False SINST: 50P: SAMPLE ID: 6 CASTOR: BD FACSDiva Software Version 8.0.1 SFL: 13088 rs SINS: 201502.014729 SPECIMEN TYPE: BM PRODUCTID: 65912.000007.2015-07-31;123456 Cells JATOS 924 CO38- cells 47.705 94.4 47.7 SSINGIES SECIISA 1.1 1000 Colls JAL Events 89.65 89.7 <	Experiment Nam	ne:	PC	ST PCD			[
Specimen Name: OneFlow PCST_001 Record Date: Jun 30, 2015 2/08:53 PM ST SETUP STATUS: SUCCESS CST SETUP TATE: 2015-02-071-2012:00:00-08:00 CST SETUP DATE: 2015-02-0711:40:33-90:00 CST PERFORMANCE EXPIRED: 2015-02-0711:40:33-90:00 CST PERFORMANCE EXPIRED: 2015-02-0711:40:33-90:00 CST REGULATORY STATUS: CE-IVD Performance Check SCF: SAMPLE ID: 6 SAMPLE ID: 6 CASE NUMBER: OG BUD: be09e04b-25e6-4b2a-ac6c-e126o4-e96846 GUD: be09e04b-25e6-4b2a-ac6c-e126o4-e96846 GUD: be09e04b-25e6-4b2a-ac6c-e126o4-e96846 SVS: Windows 7 6.1 SVS: Windows 7 6.1 SPECIMEN TYPE: BM PRODUCT ID: 659312,000007:2015-07-31;123456 Cells A1E Veents 98;05	Plate Name:								
Tube Name: OneFlow PCST_001 Record Date: Jun 30, 2015 208:53 PM CST SETUP STATUS: SUCCESS CST BEADS LOT ID: 42248 CYTOMETER CONFIG REATE DATE: 2015-02:0074-02400 CST SETUP STATUS: 2015-02:00712:40:54-08:00 CST SETUP DATE: 2015-02:007112:40:54-08:00 CST PERFORMANCE EXPIRED: 2015-02:01714:03:39-08:00 CST PERFORMANCE EXPIRED: 2015-02:01712:40:54-08:00 CST PERFORMANCE EXPIRED: 2015-02:01712:40:54-08:00 CST PERFORMANCE EXPIRED: 2015-02:01712:40:54-08:00 CST PERFORMANCE EXPIRED: False SINST: SOP: SAMPLE ID: 6 CASE NUMBER: 06 OUD: be09e04b:2566-4b:2a:ac6c=12604=968:46 CREATOR: BD FACSDixe Software Version 8.0.1 SYS: Windows 76.1 SYS: Windows 76.1 SYS: Windows 76.1 SYS: UVM Population Parent %Grand Parent %Total All Events 39.695 89.7 #### Stocial <td>Specimen Name</td> <td>e:</td> <td>On</td> <td>eFlow PCST_</td> <td>_001</td> <td></td> <td></td> <td></td> <td></td>	Specimen Name	e:	On	eFlow PCST_	_001				
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Cs1 SE IOP DATE: 2015-02-091124/03-408:00 CST BASELINE DATE: 2015-01-201714:033-408:00 CST PERFORMANCE EXPIRED: 2015-01-201714:033-408:00 CST REGULATORY STATUS: CE-IVD Performance Oteck CST REGULATORY STATUS: CE-IVD Performance Oteck SOP: SAMPLE ID: SOP: 6 CASE NUMBER: 06 GUID: be09e04b-25e6-4b2a-ac6c-e126c4e96846 CREATOR: BD FACSDix Software Version 8.0.1 SYS: Windows 7.6.1 SETTINGS: 201500/2014/729 SPECIMEN TYPE: BM PRODUCT ID: 65912.000007/2015-07-31;123456 DOCTOR: DVM Opulation Parent Name #Events %Parent %Grand Parent %Total All Events 89.695 89.7 #### SSG Singlets FSC Singl. 82.90 0.3 0.2 Plasma Cells A205 97.9 92.4 82.9 CO38+ cells SSC Singlets SSC Singlets 92.5 1.0 Plasma Cells	CYTOMETER CC	ONFIG CREATE DAT	E: 20	07-01-02112:	00:00-08:00				
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Call Performance Check 2019/02/10112/403-90.00 CST REGULTORY STATUS CE-IVD Performance Check CST BEADS EXPIRED: False SINST: SOP: SAMPLE ID: 6 ACSE NUMBER: 06 GUID: be09e04b-25e6-4b2a-ac6c-e126o4e96846 CREATOR: BD FACSDix Software Version 8.0.1 SYS: Windows 7 6.1 SYTNS: 20150629144729 SPECIMEN TYPE: BM PRODUCTID: 659312.00007.2015-07-31;123456 OFXPLATE VERSION ID: PCSTV1.0 DOCTOR: DVM Opulation Parent Name #ItEvents \$9,Parent %Grand Parent %Total All Events #### 100.00 Colls All Events \$9,000 \$9,79 SSC Singlets S20,905 \$9,79 \$9,24 \$2,90 OD38+ cells S20,50 \$9,79 \$9,24 \$2,90 OD38+ cells S20,50 \$7,90 \$2,4 \$2,90 Opg/ch- Plasma Cc 102 <t< td=""><td>COT DEDEODM</td><td></td><td>20</td><td>15-01-20114: 15-02-10T12</td><td>03:39-08:00</td><td></td><td></td><td></td><td></td></t<>	COT DEDEODM		20	15-01-20114: 15-02-10T12	03:39-08:00				
Cost READS EXPIRED: False SOP: SoP: SAMPLE ID: 6 ALL SCHEMER: 06 GUID: be09e04b-25e6-4b2a-ac6c-e12604e96846 GUID: be09e04b-25e6-4b2a-ac6c-e12604e96846 GRUD: be09e04b-25e6-4b2a-ac6c-e12604e96846 CREATOR: BD FACSDiva Software Version 8.0.1 SYS: Windows 76.1 SETTINOS: 20150629144729 SPECIMEN TYPE: BM PRODUCTID: 659912.000007.2015-07-31.123456 TEMPLATE BUILD: 06/232015 TEMPLATE BUILD: 06/232015 OCTOR: DVM Population Parent Name AIL Events 39.695 S9.7 #### SSC Singlets S2.095 Colis AIL Events S9.695 39.7 S9.7 #### SSC Singlets S2.095 Colis AIL Events S9.695 39.7 S9.7 #### S9.7 #### S9.7 <td>CST PEGULATO</td> <td>DV STATI IS</td> <td>20</td> <td>IVD Perform</td> <td>40.34-08.00</td> <td></td> <td></td> <td></td> <td></td>	CST PEGULATO	DV STATI IS	20	IVD Perform	40.34-08.00				
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OWPLE ID: 6 OASE NUMBER: 06 CASE NUMBER: 06 GUID: be09e04b-25e6.4b2a-ac6c-e126c4e98846 CREATOR: BD FACSDiva Software Version 8.0.1 SFIL: 13088 fca SYS: Windows 7 6.1 SETTINGS: 20150629144729 SPECIMEN TYPE: BM PRODUCT ID: 659912,00007,2015-07-31;123456 OE232015 TEMPLATE BUILD: DOCTOR: DVM Population Parent Name #Events 100.00 Cells A1 Events SS Singlets Cells SS Singlets S2.015 Colls S2.905 S97.9 92.4 SS Singlets S2.005 Colls SS2.51.2 O134 rotts SS2.5 Vig/4 Plasma C.1 Ord/K* Plasma C.1 Ord/K* Plasma C.1 S42.9 0.3 Colls 2.51.2	SOP								
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GUID: be09e04b-25e6-4b2a-ac6c-e126c4e96846 CREATOR: BD FACSDive Software Version 8.0.1 \$FIL: 13088 fca SYS: Windows 7 6.1 SETTINGS: 20150629144729 SPECIMEN TYPE: BM PROUDOT ID: 659912.000007.2015-07-31;123456 TEMPLATE BVILD: 06232015 TEMPLATE VERSION ID: PCSTV1.0 DOCTOR: DVM Opulation Parent Name #LEvents 98,695 SSINglets ECS Singlets Colls AL708 SSC Singlets S20,905 Plasma Cells 203,003 OD34: cells S20,905 Plasma Cells CD38: cells SSC Singlets S22,905 Plasma Cells CD34: cells OydyL+ Plasma C. Vig4.+ Plasma C. Vig4.+ Plasma C. Vig4.+ Plasma C.	CASE NUMBER: 06								
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Plasma Cells CD38-cells 243 98.0 0.3 0.2 cylp/+ Plasma C 127 52.3 51.2 0.1 cylg/+ Plasma C 106 44.4 43.5 0.1	CD38+ celle	SSC Singl	248	0.3	0.3	0.2			
Origit Plasma C 127 52.3 51.2 0.1 Origit+ Plasma C 108 44.4 43.5 0.1 Origit+ Plasma C 108 44.4 43.5 0.1	Plasma Cells	CD38+ cells	243	98.0	0.3	0.2			
cylgL+ Plasma C 108 44.4 43.5 0.1	cylaK+	Plasma C	127	52.3	51.2	0.1			
Deally 1645 20 10 16	cylal +	Plasma C	108	44.4	43.5	0.1			
5 Cells 53C Sindi 1,043 2.0 1.9 1.0	Bcells	SSC Singl	1,645	2.0	1.9	1.6			

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	1.	From the menu bar, select File > Import > Experiments.
BD OneFlow PCD	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.

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DOCTOR:			DVM			
TEMPLATE BUIL	D:		06/22/15			
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CD38+ cells	SSC Singlets	213	0.3	0.3	0.2	
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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	You may observe that templates do not import correctly. For
import correctly	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 (Ιgκ and Ιgλ) 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.	 Navigate to the analysis worksheet. Right-click the statistics box and select Edit Stats View. In the Header tab, select the All checkbox. 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.	 From the BD FACSDiva software menu bar, select File > Page Setup. Ensure that all of the margins are set to 2.54 cm or 1 inch, depending on your default standards. Click OK.

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