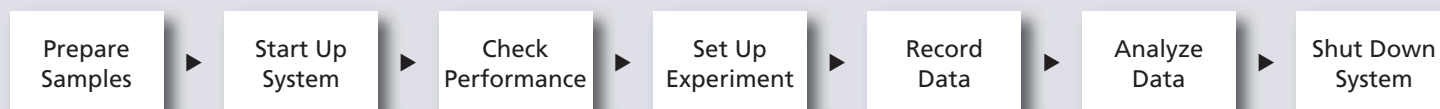


BD FACSCanto Clinical Software Quick Reference Guide for BD FACSCanto Systems with Loader Option

This guide contains instructions for using BD FACSCanto™ clinical software with BD FACSCanto and BD FACSCanto II systems with the BD FACSTM Loader option. The workflow shown uses the BD FACSTM Sample Prep Assistant III (SPA III) to prepare lyse/no-wash samples. Before starting your daily workflow, ensure that your lab's software administrator has performed all the necessary tasks to set up the SPA III and BD FACSCanto clinical software for your use.

Workflow Overview

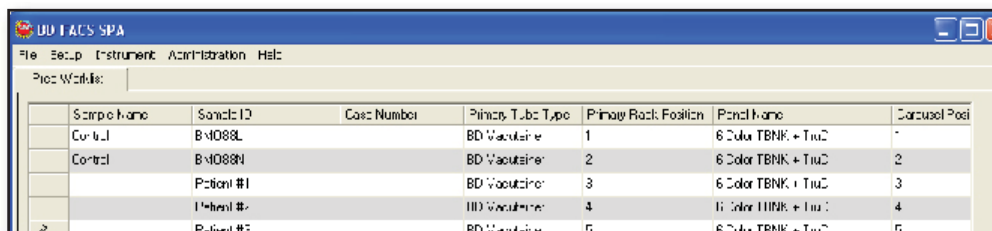
The following figure shows the steps for the daily workflow using BD FACSCanto software.



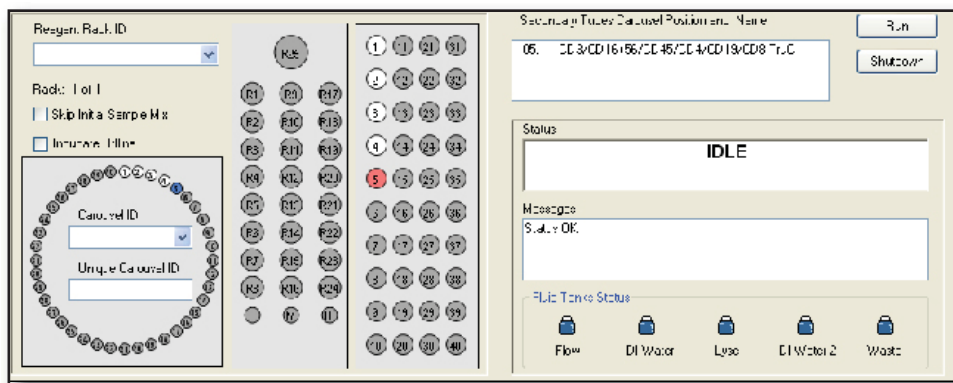
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Preparing Samples on the SPA III

- 1 Perform daily inspection and startup for the SPA III instrument.
- 2 Set up the worklist in the BD FACSTM SPA software.



- 3 Load the primary tubes in the primary tube rack, secondary tubes into the carousel, and reagents into the reagent rack as specified in the software.



- 4 Close the safety cover and click **Run** to process samples.
- 5 Save and print the SPA worklist.
- 6 Transfer the SPA worklist to a location where it can later be imported into BD FACSCanto clinical software.
- 7 Perform daily cleaning.
- 8 Shut down the SPA III.

Starting Up the System

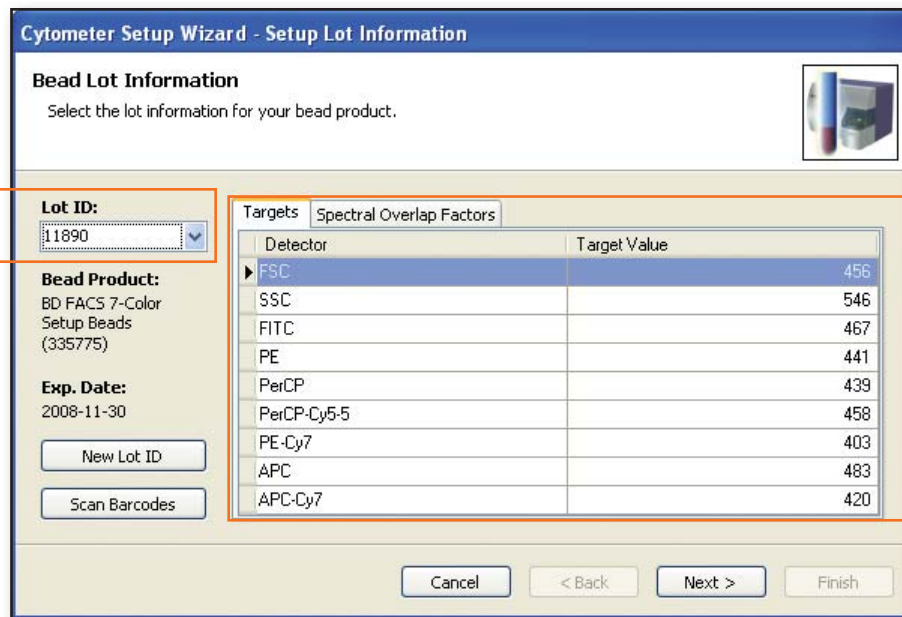
- 1 Turn on the cytometer main power.
- 2 Start up the computer, start BD FACSCanto clinical software, and log in.
- 3 Check cytometer conditions in the Status window.
- 4 Select **Cytometer > Fluidics Startup** if automatic cleaning is disabled.
- 5 Check the flow cell for air bubbles.
- 6 Check that laser warmup has finished, indicated by a ready status.



TIP Allow the lasers to warm up for 15 to 30 minutes before running samples on the cytometer to ensure laser stability and optimal power.

Checking Cytometer Performance

- 1 Select **Cytometer > Setup > Standard Setup**.



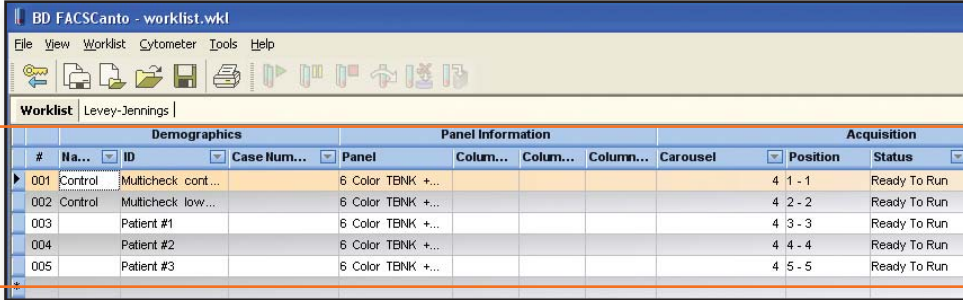
Detector	Target Value
FSC	456
SSC	546
FITC	467
PE	441
PerCP	439
PerCP-Cy5-5	458
PE-Cy7	403
APC	483
APC-Cy7	420

- 2 Place a tube of the BD FACSTM 7-color setup beads in position 1 on a carousel and run the beads.
- 3 View the Cytometer Setup Report.
- 4 If setup passes, close the setup report and click **Finish**.
If setup fails, refer to the Setup Troubleshooting table in the BD FACSCanto Clinical Software Help system.

Setting Up the Experiment and Recording Data

- 1 Import the SPA worklist.

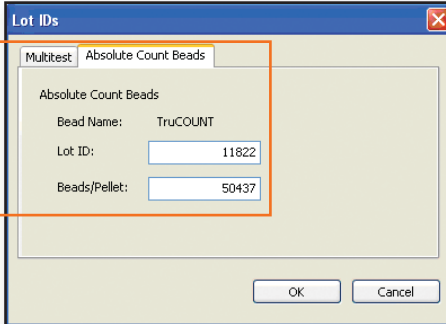
Review the imported information and edit missing or incorrect entries if needed.



Demographics				Panel Information			Acquisition			
#	Na...	ID	Case Num...	Panel	Column...	Column...	Column...	Carousel	Position	Status
001	Control	Multicheck cont...		6 Color TBNK +...				4	1 - 1	Ready To Run
002	Control	Multicheck low...		6 Color TBNK +...				4	2 - 2	Ready To Run
003		Patient #1		6 Color TBNK +...				4	3 - 3	Ready To Run
004		Patient #2		6 Color TBNK +...				4	4 - 4	Ready To Run
005		Patient #3		6 Color TBNK +...				4	5 - 5	Ready To Run

- 2 Enter lot IDs.

Enter lot IDs for reagents and beads as needed.



Lot IDs

Multitest: Absolute Count Beads

Absolute Count Beads

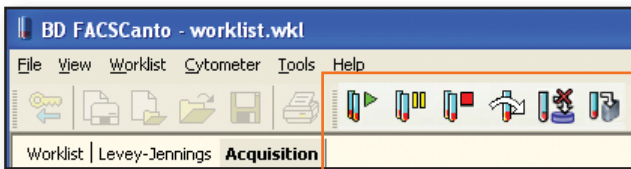
Bead Name: TruCOUNT


Lot ID: 11822



Beads/Pellet: 50437

OK Cancel

- 3 Vortex sample tubes prior to placing the carousel on the Loader.
- 4 Verify that cytometer settings are appropriate, then record data.



Click **Run** () to start acquisition.

If needed, click **Pause** () and then **Optimize** () to optimize cytometer settings.

After optimization, click **Run** to return to data recording. Apply changes to a tube, a sample, or all samples in the worklist.

NOTICE: If you optimize cytometer settings, the settings from the .opt file are automatically applied to future samples when the same panel is chosen. See the *BD FACSCanto II Instructions for Use* for information about maintaining optimization settings.

Analyzing Data

- 1 View the Lab Report for each sample.

Double-click in the Status field to view a report.

Status	FCS File
OK	Multicheck control00...
OK	Multicheck low contro...

Make adjustments to the gates as needed.

Parameter	Percent	Value/AbsCnt
Lymph Events		2576
Bead Events		1682
CD3+	70.77	1098.57
CD3+CD8+	20.23	313.96
CD3+CD4+	49.69	771.35
CD3+CD4+CD8+	0.78	12.05
CD16+CD56+	11.76	182.59
CD19+	15.49	240.44
CD45+		1552.34
4/8 Ratio		2.46

QC Messages
Manual Gate is in effect.
% T-Sum is: 0.85
Lymphsum is: 98.02
4/8 ratio is: 2.46

Comments

- 2 Verify that a report has been reviewed as needed.
- 3 Click the **Worklist** tab and continue reviewing Lab Reports.
- 4 Print all Lab Reports as needed.
- 5 Save the worklist.
- 6 Exit BD FACSCanto clinical software.

Shutting Down the System

Use BD FACSDiva™ software to perform automatic cleaning using the Loader.

- 1 Start BD FACSDiva software and log in.
- 2 Verify that the flow rate in the **Acquisition Dashboard** is set to **Medium** or **High**.
- 3 Select **Carousel > Clean**.

Clean with carousel tubes...

Selections and Settings

Tubes	Time (min)
<input checked="" type="checkbox"/> Cleaning	5
<input checked="" type="checkbox"/> Rinse 1	5
<input checked="" type="checkbox"/> Rinse 2	5

Select cleaning tubes and time.

OK Cancel

- 4 Install the carousel with the appropriate cleaning tubes and perform the cleaning cycle.
- 5 Perform a fluidics shutdown.
- 6 Empty waste and refill fluids if prompted to do so.
- 7 Turn off the cytometer main power and shut down the computer.