**BD FACS CALIBUR PROTOCOL**

Last updated by J Le Blanc, J Rogers, K Haley, and H Qin on 2012 June 1.

**Turning ON**

1. Sign the starting time, user name on the log book.
2. Empty waste container on the right side of machine (unscrew white lid and decant waste)
3. Check sheath fluid level in the source (container on left side) to see if it is at proper level; which is at the black line.
4. If sheath fluid level is not at proper level, use funnel-cylinder to collect sheath fluid, unscrew left container top to fill until the fluid is at proper level.
5. Once filled to black line, screw source top back into the container.
6. Turn black switch down to “Pressurize run” before turning on machine.
7. Remove bubbles in tubes (press prime, run, and then hi); use scroller to remove bubbles.
8. Turn on green power button on right side of machine.
9. Turn on computer
10. Log-in to computer as ‘hqin’ and password

**Turning OFF**

1. Move base lever (SIPPER) to the right to remove used BD tube, do so quickly.
2. Fill a BD tube with 10% bleach and place the tube in the designated tube area
3. Leave the base lever unfastened for 1 minute (with the buzzing sound)
4. Switch the base lever undeath the BD tube (turn it to the left; Press “Run” and “High”) and let the bleach sit for 5 minutes.
5. After 5 minutes, remove the 10% bleach BD tube by moving base lever to the right and press the “standby” button.
6. Move base lever back to center
7. Throw away BD Tube with 10% bleach away in trash can
8. Fill another BD Tube with ddH2O and place in the designated tube area
9. Leave the base lever unfastened for 1 minute on a standby mode (with buzzing sound and keep eyes on the level of water in a tube)

Fasten the base lever (turn it to the left; Press “Run” and “High”) and let the ddH2O sit for 5 minutes.

1. Switch off machine by pressing the green button on right side of machine.
2. Switch black lever back to “Vent- Change Tubes”
3. Write the finish time on the log-book and status of the instrument and computer. Unscrew waste can top and decant used sheath fluid in sink.

**OK, Please write some procedure on running the machine here, - HQIN**

**Notes:**

**The computer is very old and can freeze for no reason during running.**

**Blank the first test.**

**If working with yeast cells, allow the counter to reach 10,000 before pausing.**

1. Double-click cell Quest

A blank template will appear, however you do not need that template.

1. Click red circle in the top left corner of the template

2. “ Don’t Save”

In order to retrieve the necessary templates:

1. File

2. Open Document

3. Click on your specific directory

4. Document

5. Lab

6. Find most recent template

7. Select Open

First, choose the appropriate template

**1.**  Vortex sample before running

1. Go to File and to Open document
2. H-Qin
3. Document
4. Lab
5. Most recent template
6. Acquire
7. Connect to Cytometer (Open, Set)
8. Setup has to be unchecked for anything to record
9. Save data in directory in lab
10. Use a new folder for every experiment

Second, Connecting computer to Calibur Instrument, choose instrument setting

1. Click Acquire (click run)
2. Connect to cytometer
3. acquisition
4. Cytometer
5. instrument setting
6. open
7. instrument setting
8. save
9. set (Wheel will begin to spin)
10. done

**For More Windows:**

**1.** Cytomer

**2.** Defectors

**3.** Cytometer

**4.** Threshold

**5.** Cytometer

**6.** Compensatio

**7.** Cytometer

**8**. Status

**Then:**

**1.** acquire

**2.** counter

**Beginning to Run Experiment:**

**\*\*Important: If “setup” is checked on acquisition control, no data will be recorded. Therefore you must uncheck “setup” in order for data to be recorded.**

**In main acquisition window:**

Directory: Where you want to save your data

File: Name of file of your current sample

**1.** Save to your specific lab

**2.** Every new experiment must have a new folder name:

a. Folder

b. Create

c. Choose

**3.** Change File Name:

a. File Name

b. Change

c. Choose

**In order to collect data Cytometer must be set on “Run” otherwise an error message will appear on monitor.**

**1. Push “Run” on cytometer**

**2. Select Acquire on monitor**

**Data should begin to appear inside charts that are open**

**Transferring Files:**

**Notes: Be sure to have a USB drive available to transfer your collected data**

You want to save the template of the instrument settings to your file to keep track of your experiment in order to compare data and also to see if something went wrong.

**1.** Click Cell Quest Pro Icon at bottom of monitor

**2.**Instrument Setting

**3.** Save

**4.** Your saved directory title

**5.** Save as

**6.** Go

Then go back to finder to be sure that the instrument setting template was saved to your lab.

**Transferring Files:**

**1.** Finder

**2.** Click on saved directory title

**3.** Drag and drop each of the saved files onto your USB drive

**4.** Correctly export USB from computer