

## **SOP: Operation of Life Technologies Countess II Cell Counter**

### **Approvals**

Preparer: Jason McMillan  
Reviewer: Dr. Maggie Bryans  
Reviewer: Hetal Doshi

Date: 09JUL15  
Date: 20DEC18  
Date: 20DEC18

### **1. Purpose**

1.1. To perform cell count and viability measurements of trypan blue stained samples.

### **2. Scope and Applicability**

2.1. Applies to the counting and viability measurement of trypan blue stained samples using the Life Technologies Countess II Cell Counter.

### **3. Summary of Method**

3.1. Turn on the Countess II Automated Cell Counter.

3.2. Mix cell aliquot with trypan blue stain.

3.3. Load 12 $\mu$ l of cell/trypan blue stain mixture into Side A of the Countess Cell Counting Chamber Slide.

3.4. Insert the Countess Cell Counting Chamber Slide into the slide port.

3.5. Perform count.

3.6. Remove the Countess Cell Counting Chamber Slide and turn around the slide, and reinsert Side B into the slide port.

3.7. Perform count.

3.8. Remove the Countess Cell Counting Chamber Slide and dispose of into the sharps container.

3.9. Load USB drive into the Countess II Automated Cell Counter and save data.

3.10. Turn off the Countess II Automated Cell Counter.

### **4. References**

4.1. Countess II/Countess II FL Automated Cell Counter Quick Reference Guide

### **5. Definitions**

5.1. N/A

### **6. Precautions**

6.1. N/A

### **7. Responsibilities**

7.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.

7.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

### **8. Equipment and Materials**

8.1. Life technologies Countess II Automated Cell Counter

8.2. Countess Cell Counting Chamber Slide

8.3. 0.4% trypan blue stain

8.4. P20 micropipette

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- 8.5. P20 micropipette tips
- 8.6. Microcentrifuge tube
- 8.7. Microcentrifuge tube rack

### **9. Procedure**

- 9.1. Turn on the Countess II Automated Cell Counter by pressing the switch on the lower, right hand corner on the back of the system.
  - 9.1.1. The power indicator light will illuminate and the 7" capacitive touchscreen will illuminate and flash the Life Technologies logo, Countess II Automated Cell Counter logo, Life technologies and Countess II Automated Cell Counter logos, and finally settling on the home screen that prompts the user to "Insert Slide."
- 9.2. Prepare sample by adding 40µl of cell suspension to 40µl of 0.4% trypan blue stain.
- 9.3. Remove a Countess Cell Counting Chamber Slide and open by tearing the wrapper at the portion labeled "Tear Here."
  - 9.3.1. Remove the Countess Cell Counting Slide and locate Side A.
- 9.4. Load 12µl of the sample mixture into Side A of the Countess Cell Counting Chamber Slide.
- 9.5. Load 12µl of the sample mixture into Side B of the Countess Cell Counting Chamber Slide.
- 9.6. Insert the Countess Cell Counting Slide, Side A first, into the slide port until you hear a soft click.
- 9.7. The instrument automatically illuminates the sample and focusses the cells.
  - 9.7.1. To enable the auto focus to function properly, manually focus the cells using the "Focus" slider and press "Set" to set the optimal focus.
  - 9.7.2. Set exposure using the "Light source" slider, and press "Capture" on the 7" capacitive touchscreen. The instrument captures the image and displays the results (total concentration, percentage and concentration of live and dead cells).
- 9.8. To count the second chamber, remove the Countess Cell Counting Slide and turn around the slide to Side B, and reinsert into the slide port until you hear a soft click.
- 9.9. To save data
  - 9.9.1. On the 7" capacitive touchscreen press "Save"
  - 9.9.2. Insert USB Drive into the USB port
  - 9.9.3. Press "Save" on the 7" capacitive touchscreen, a check mark with the phrase "Save Complete" will appear.
  - 9.9.4. On the 7" capacitive touchscreen press "Close."
  - 9.9.5. Remove USB drive
- 9.10. Remove the Countess Cell Counting Slide and dispose of in a sharps container.
- 9.11. Turn off the Countess II Automated Cell Counter by pressing the switch on the lower, right side on the rear of the instrument.

### **10. Attachments**

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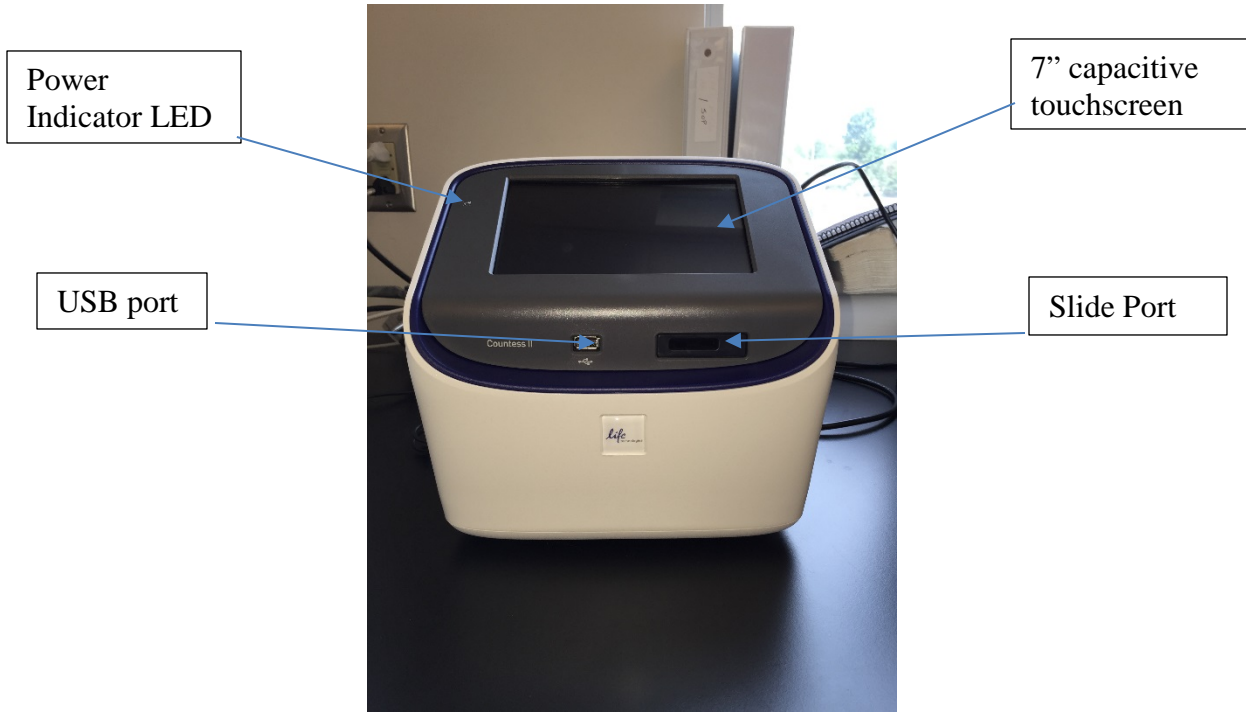


Figure 1. Countess II Cell Counter front panel

### 11. History

<i>Revision Number</i>	<i>Effective Date</i>	<i>Preparer</i>	<i>Description of Change</i>
0	09JUL15	Jason McMillan	Initial release
1	20DEC18	Hetal Doshi	Change the volume of sample and trypan blue to 40µl and Sample loading volume to 12µl