

SOP: Operation of Logos biosystems Luna-FL Fluorescence Cell Counter for Fluorescence Cell Counting

Approvals:

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Date: 07Jul21

Reviewer: Hetal Doshi

Date: 08Jul21

Reviewer: Juan Perez Rodriguez

Date: 12Jul21

Reviewer: Maggie Bryans

Date: 12Jul21

1. Purpose:

- 1.1. To perform cell count and viability measurements of cell culture samples.

2. Scope:

- 2.1. This SOP applies to the preparation of samples and determining the cell count and cell viability of the sample using Acridine Orange/Propidium Iodide Stain and the Luna-FL cell counter.

3. Summary of Method:

- 3.1. Turn on the Luna-FL cell counter
- 3.2. Clean reusable slide
- 3.3. Mix cell culture sample with Acridine Orange/Propidium Iodide stain
- 3.4. Load sample on to the Luna reusable slide
- 3.5. Place the prepared slide in the slide port of the counter
- 3.6. Perform the count and save the results
- 3.7. Remove the slide
- 3.8. Clean the reusable slide

4. Responsibilities:

- 4.1. 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.
- 4.2. 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.

5. References:

- 5.1. Luna-FL Automated Fluorescence Cell Counter Quick Start Guide
- 5.2. Luna-FL Automated Fluorescence Cell Counter User Manual
- 5.3. Luna Reusable Slide from Logos Biosystems PDF
- 5.4. Luna Reusable Slide cleaning <https://www.youtube.com/watch?v=a-EZmlbQVvk>

6. Precautions:

- 6.1. Routine care should be exercised in the handling of cell samples and stains, which may have harmful biological activity in the case of accidental exposure.
- 6.2. Gloves, a lab coat and protective eyewear should be worn.
- 6.3. Always wear gloves when handling the Luna reusable slide.

7. Materials:

- 7.1. Luna-FL Automated Fluorescence cell counter
- 7.2. Luna reusable slide and coverslip
- 7.3. Acridine Orange/Propidium Iodide stain

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- 7.4. Cell sample to count
- 7.5. P20 micropipette and tips
- 7.6. Microcentrifuge tubes
- 7.7. Microcentrifuge tube rack
- 7.8. MilliQ water squirt or spray bottle
- 7.9. 70% ethanol squirt or spray bottle
- 7.10. Lint free laboratory wipes

8. Procedure:

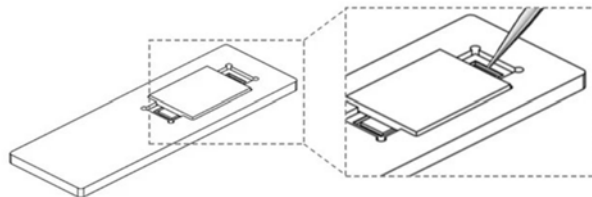
- 8.1. Turn on the Luna-FL cell counter using the power switch on the back lower left. The start-up screen will be displayed. (Attachment 9.3 Home Screen)
- 8.2. Prepare the Luna reusable slide
 - 8.2.1. Holding the slide firmly by the edges over a sink flush the chamber with MilliQ water using either a squirt or spray bottle. Gently shake the excess water from the slide. Do not soak the slide in water.
 - 8.2.2. Wipe the slide chamber using a lint-free laboratory tissue using long swipes do not scrub.
 - 8.2.3. Holding the slide firmly by the edges over a sink rinse the chamber with 70% ethanol using either a squirt or spray bottle. Gently shake the excess ethanol solution from the slide.
 - 8.2.4. Wipe the slide chamber using a lint-free laboratory tissue using long swipes. Do not scrub.
 - 8.2.5. Clean the coverslip using the same steps used for cleaning the slide.
 - 8.2.6. Place the coverslip over the sample chamber of the slide.
- 8.3. Prepare the sample to be counted
 - 8.3.1. Add 2 μ l of the Acridine Orange/Propidium Iodide stain to a new 1.5ml microfuge tube.
 - 8.3.2. Add 18 μ l of the cell sample to the microfuge tube and mix by pipetting up and down or flicking the bottom of the tube.
- 8.4. Load 12 μ l of the prepared sample on to the Luna reusable slide.
 - 8.4.1. Tilt the pipette at a 45 to 60° angle and place the pipette tip into the space between the slide and the coverslip at the edge of the chamber nearest the arrow. (see Attachment 9.2) Fill with 12 μ l of sample cell suspension. Be careful not to lift the coverslip with the pipette tip.
- 8.5. Perform the cell count and viability measurements;
 - 8.5.1. Insert the slide arrow side first, coverslip up into the slide port on the right-hand side of the Luna cell counter.
 - 8.5.2. On the touch screen select, “Fluorescence Cell Counting”. The preview image of the cell sample will be displayed on the screen. (Attachment 9.3 “Bright Field mode in Fluorescence Counting” screen)
 - 8.5.3. Wait for 10 seconds to 30 seconds for the cells to settle down.
 - 8.5.4. Use the focus knob, on the right side of the instrument, to focus the sample image. The live cells should have bright centers and crisp dark edges.
 - 8.5.5. On the touch screen select, “Bright Field”,
 - 8.5.6. On the “Green Fluorescence mode in Cell Counting” screen, (Attachment 9.3), adjust the level of exposure.

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- 8.5.6.1. Set the exposure to either 6 or 7 using the up and down arrows on the lower left corner of the touch screen. The image is too dim if not all the cells are visible and too bright if there is any background fluorescence.
- 8.5.6.2. Work quickly to make this adjustment to avoid bleaching of the cells.
- 8.5.7. Make sure that the cells have settled down before pressing the “Count” button.
- 8.5.8. Press “Count”. Within approximately 30 seconds the image of the cell sample and the following results will be displayed on the screen; Total cells, Live cells, Dead cells, Viability, average size, total number, live number and dead number.
- 8.6. Record the data or Save your results to a USB drive in the USB port on the left side of the instrument.
 - 8.6.1. Press the “Save/Print” button on the touch screen a new, “Save” window appears. The red check boxes at the top of the screen indicate what data will be saved.
 - 8.6.2. Enter a file name for the results to be saved using the keyboard buttons. Add date and time using “Date/Time” button. Press “Enter” to save the results to the USB drive.
- 8.7. Remove the Luna reusable slide from the slide port.
- 8.8. Clean the Luna reusable slide
 - 8.8.1. Gently remove the coverslip from the slide and place on a lint free laboratory tissue.
 - 8.8.2. Holding the slide firmly by the edges over a sink flush the chamber with MilliQ water using either a squirt or spray bottle. Gently shake the excess water from the slide. Do not soak the slide in water.
 - 8.8.3. Wipe the slide chamber using a lint-free laboratory tissue using long swipes do not scrub.
 - 8.8.4. Holding the slide firmly by the edges over a sink rinse the chamber with 70% ethanol using either a squirt or spray bottle. Gently shake the excess ethanol solution from the slide.
 - 8.8.5. Wipe the slide chamber using a lint-free laboratory tissue using long swipes. Do not scrub.
 - 8.8.6. Clean the coverslip using the same steps used for cleaning the slide.
 - 8.8.7. Return the clean slide and coverslip to the storage box when finished.
- 8.9. Turn off the Luna-FL Cell Counter.

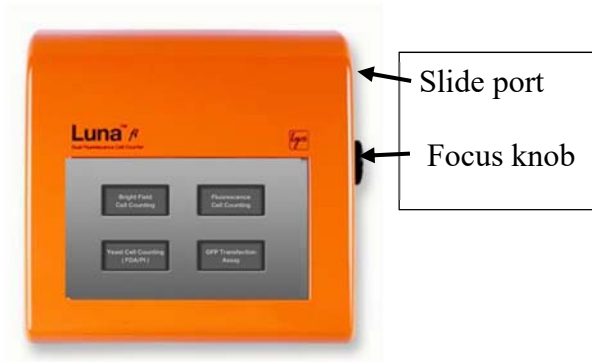
9. Attachments

9.1 loading the Luna reusable slide

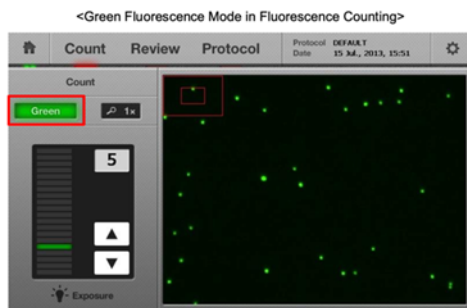
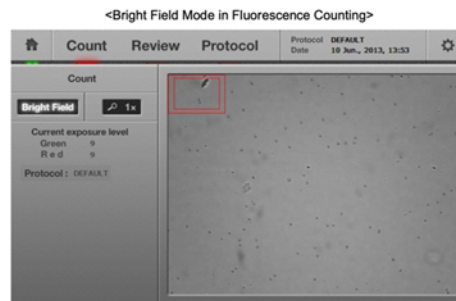
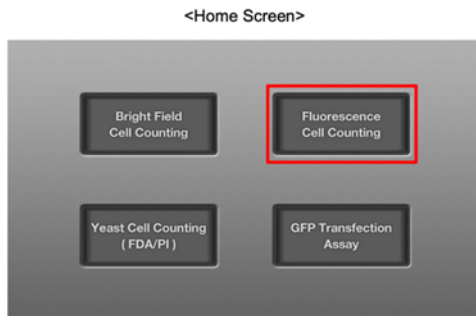


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9.2 Luna-FL cell counter



9.3 Luna-FL data display screens



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Fluorescence Cell Counting**

10. History:

Revision Number	Effective Date	Preparer	Description of Change
0	13Jul21	Robin Zuck	Initial release