

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

Approvals:

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Date: 07Jul21

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Date: 08Jul21

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Date: 12Jul21

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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 or use disposable 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. Disposable Luna slide (PhotonSlide, compatible for fluorescence cell counting)

7.4. Acridine Orange/Propidium Iodide stain

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- 7.5. Cell sample to count
- 7.6. P20 micropipette and tips
- 7.7. Microcentrifuge tubes
- 7.8. Microcentrifuge tube rack
- 7.9. MilliQ water squirt or spray bottle
- 7.10. 70% ethanol squirt or spray bottle
- 7.11. 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 9.4 Home Screen) Proceed to step 8.2 for using a Luna reusable slide and to step 8.10 for using a Luna disposable slide.
- 8.2. ***Cell counting with a 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",

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- 8.5.6. On the “Green Fluorescence Mode” in the cell counting screen, (Attachment 9.4), adjust the level of exposure.
 - 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.
 - 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 cell concentration, live cell concentration, dead cell concentration, viability, average cell size, total cell number, live cell number, and dead cell 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.
- 8.10. ***Cell counting with a Luna disposable slide.***
 - 8.10.1. Tear off the top of the wrap and gently take out the slide (**Note: Do not press down a slide with or without wrapping. When handling, hold the edges of the wrapping, not the slide itself, widen the opening and gently remove the slide to prevent possible scratches. Avoid fingerprints on the chamber area**)
 - 8.10.2. Prepare the sample to be counted by following steps in section 8.3.
 - 8.10.3. Load 12 μ L of sample to one side chamber. There are two chambers for loading samples (Attachment 9.2)
 - 8.10.4. Insert the slide in such a way that the chamber containing the cell sample is inserted first.
 - 8.10.5. Perform the cell count by following steps 8.5.2. to 8.5.8.
 - 8.10.6. Record the data or save results on USB drive by following steps in section 8.6.
 - 8.10.7. Remove the slide.

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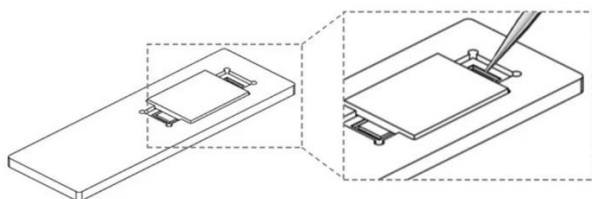
8.10.8. Save slide if both chambers have not been used.

8.10.9. Dispose of slide in biohazard waste bin when both chambers have been used.

8.10.10. Turn off the Luna-FL Cell Counter

9. Attachments

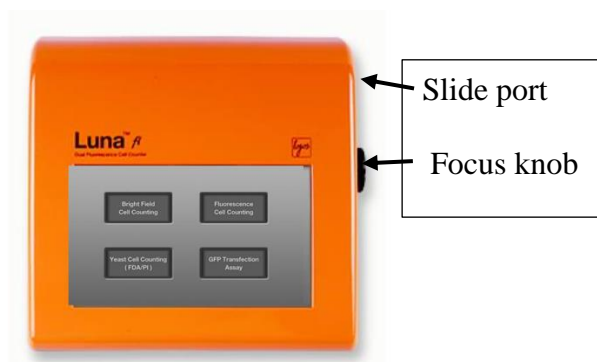
9.1 loading the Luna reusable slide



9.2. Loading the Luna disposable slide

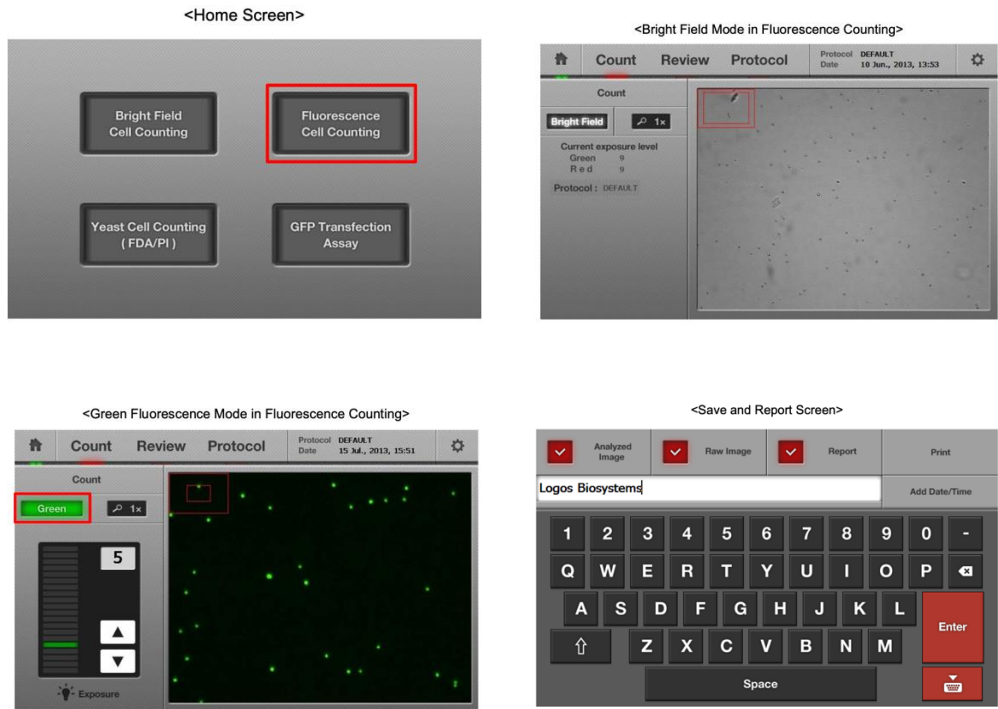


9.3. Luna-FL cell counter



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9.4 Luna-FL data display screens



10. History:

Revision Number	Effective Date	Preparer	Description of Change
0	13Jul21	Robin Zuck	Initial release
1	20Jul23	Hetal Doshi	Added steps for performing cell count using disposable slide