



User Manual



RÍ

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DISCLAIMER

The contents of this document are subject to change without notice. The LUNA-FX7™ Automated Cell Counter is an electrical laboratory instrument for scientific research use only. It is not a medical, therapeutic, or in vitro diagnostics device. Do not disassemble the device on any occasion as this will invalidate your warranty.

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TRADEMARKS

enetics, inc.

CERTIFICATION MARKS

X	The WEEE (Waste Electrical and Electronic Equipment) symbol indicates that users of this instrument have responsibility of returning and disposing of WEEE in an environmentally friendly manner. Follow the waste ordinances of your region for proper disposal provisions.
CE	The CE mark indicates that this instrument conforms to all applicable European Community provisions for which this marking is required. Users must be aware of and follow the conditions described in this manual for operating the instrument. The protection provided by the instrument may be impaired if the instrument is used in a manner not specified by this manual.
	Protective earth (Ground)
FC	This device complies with Part 15 of the FCC Rules.
C SUD US	This equipment complies with the requirement of UL 61010-1:2012, CAN/CSA C22.2 No.61010- 1:2012. "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements."
K	The KC certification mark indicates that this instrument conforms with Korea's product safety requirements for electrical and electronic equipment and components for which this marking is required.

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Safety Precautions

Instrument Safety

General Safety Operate the instrument in the conditions described in the Operating Conditions.

Install the instrument on a level and sturdy surface. Avoid vibrations from other devices.

Do not touch components with wet hands.

Use components provided or authorized by Logos Biosystems. If the proper combination of components is not used, product safety cannot be guaranteed.

Use only the power cord and AC adapter provided by Logos Biosystems. If the proper power cord and AC adapter are not used, electrical safety of the product cannot be guaranteed.

Ensure that the input voltage is compatible with the power supply voltage of the product.

Connect the grounding terminal of the instrument and electrical outlet properly. If the instrument is not grounded, electrical safety of the product cannot be guaranteed.

Turn on the instrument only after connecting the power cord and AC adapter to both the power source and the instrument. Turn off the instrument before disconnecting the power cord and/or moving the instrument.

Disconnect the power cord in the case of abnormalities.

Be careful with possible electric shock hazards as electric current may be still alive when the instrument stops.

Do not hold the slide slot while it is in motion.

Protect USB drives from being infected with viruses and malware.

Before shutting down or moving the device, remove the slides from the slide holder. If slides are left inside the slide holder, they may fall into the internal parts of the equipment and cause malfunctions.

Frequency 50/60 Hz Electrical Input 12 VDC, 5.0 A Installation Site Indoor use only Operating Temperature 10 - 35°C Maximum Relative Humidity 10 - 80%	Operating	Operating Power	100 - 240 VAC, 1.5 A		
Installation Site Indoor use only Operating Temperature 10 - 35°C	Conditions	Frequency	50/60 Hz		
Operating Temperature 10 - 35°C		Electrical Input	12 VDC, 5.0 A		
		Installation Site	Indoor use only		
Maximum Pelative Humidity 10 - 80%	Operating Temperature		10 - 35°C		
		Maximum Relative Humidity	10 - 80%		
Altitude ≤ 2,000 m	Altitude		≤ 2,000 m		

Instrument

Follow the rules and regulations of your local government.

Disposal

Do not disassemble the instrument in any event as this will invalidate your warranty.

Disassembly

Instrument

If the instrument is damaged or malfunctioning, contact your local distributor or Logos

Personal Safety

Biosystems.

Safety Guidelines Read and understand all user manuals thoroughly before using the instrument.

Keep all user manuals in a safe and accessible place for future reference.

Read and understand all safety data sheets before storing, handling, or working with any reagents.

Wear appropriate personal protective equipment (PPE) when handling reagents and cell samples to avoid exposure.

When using toxic agents, radioactive materials, or pathogenic microorganisms belonging to WHO Risk Groups 2-4, follow national laws and regulations for biosafety level requirements.

This instrument is to be serviced by trained personnel only to avoid injury.

<text><text> Do not reuse disposable slides. Used slides must be disposed as biohazardous waste

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Précautions de sécurité

Sécurité des instruments

Sécurité générale Faites fonctionner l'instrument dans les conditions décrites dans les conditions de fonctionnement.

Installez l'instrument sur une surface plane et solide. Évitez les vibrations provenant des autres appareils.

Ne touchez pas les composants avec les mains mouillées.

Utilisez uniquement les composants fournis ou autorisés par Logos Biosystems. En cas d'utilisation d'une combinaison autre que celle qui a été recommandée, la sécurité du produit ne peut être garantie.

Utilisez uniquement le cordon d'alimentation et l'adaptateur fournis par Logos Biosystems. En cas d'utilisation du cordon et de l'adaptateur non appropriés, la sécurité electrique du produit ne peut être garantie.

Assurez que la tension d'entrée est compatible avec la tension d'alimentation du produit.

Connectez correctement la borne de mise à la terre de l'instrument et la prise électrique. Si l'instrument n'est pas mis à la terre, la sécurité électrique du produit ne peut pas être garantie.

Allumez l'instrument uniquement après avoir connecté respectivement le cordon d'alimentation et l'adaptateur à la source d'alimentation et à l'instrument. Éteignez l'instrument avant de débrancher le cordon d'alimentation et / ou de déplacer l'instrument.

Débranchez le cordon d'alimentation en cas d'anomalies.

Soyez prudent avec les risques d'électrocution, car le courant électrique peut être encore actif lorsque l'instrument s'arrête.

Ne tenez pas le tiroir de lame lorsqu'elle est en mouvement.

Protégez les clés USB contre les virus et les logiciels malveillants.

Avant d'éteindre ou de déplacer l'appareil, retirez les lames du support de lame. Si des lames restent à l'intérieur du support de lame, elles peuvent tomber sur des pièces internes de l'équipement et causer des dysfonctionnements.

Conditions de fonctionnement

Puissance de fonctionnement	100 - 240 VAC, 1.5 A		
Fréquence	50 / 60 Hz		
Entrée électrique	12 VDC, 5.0 A		
Site d'installation	Utilisation en intérieur uniquement		
Température de fonctionnement	10 - 35°C		
Humidité relative maximale	10 - 80%		
Altitude	≤ 2,000 m		

Destruction de l'instument

Suivez les règles et réglementations de votre gouvernement local.

Démontage de l'instrument

Ne démontez en aucun cas l'instrument car cela invaliderait votre garantie. Si l'instrument est endommagé ou fonctionne mal, contactez votre distributeur local ou Logos

Sécurité personnelle

Biosystems.

Consignes de Lisez et comprenez attentivement tous les manuels d'utilisation avant d'utiliser l'instrument. sécurité Conservez tous les manuels d'utilisation dans un endroit sûr et accessible pour référence future. Lisez et comprenez toutes les fiches de données de sécurité avant de stocker, de manipuler ou de travailler avec des réactifs. Porter un équipement de protection individuelle (EPI) approprié lors de la manipulation des réactifs et des échantillons cellulaires pour éviter toute exposition. Lors de l'utilisation d'agents toxiques, de matières radioactives ou de micro-organismes pathogènes appartenant aux groupes de risque 2 à 4 de l'OMS, respectez les lois et réglementations nationales relatives aux exigences de niveau de biosécurité. Cet instrument doit être entretenu par du personnel qualifié uniquement pour éviter les blessures. Traitement des Ne réutilisez pas les lames jetables. Les lames usagées doivent être éliminées comme des déchets biodangereux conformément aux règles et réglementations de votre gouvernement déchets local.

1. Product Introduction

Product Contents

Component Quantity LUNA-FX7™ Automated Cell counter 1 Power Cord with AC Adapter 1 2 ea / 4 slide types Cell Counting Slides Sample Trypan Blue Stain, 0.4 % 2 x 1 mL Acridine Orange/Propidium Iodide Stain 2 x 0.5 mL LUNA-FX[™] Calibration Beads Kit 1 WiFi Dongle 1 1 USB Drive Installation Guide 1 Quick Start Guide 1

Product Contents The LUNA-FX7[™] Automated Cell Counter is shipped with the following components.

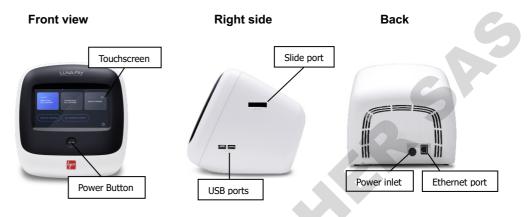
Inspect the product package upon delivery to ensure that all components have been included. Contact your local distributor or Logos Biosystems if anything is missing. Damage that may occur during shipping and handling is not covered by warranty and must be filed with the carrier.

Product Description

LUNA-FX7™ Automated Cell

Counter

The LUNA-FX7[™] is an automated, image-based cell counting device that features an accurate counting algorithm and increased counting volume and represents a fully automated solution for cell counting and viability analysis. The LUNA-FX7[™] also provides flexible counting slide options from a single channel slide to a higher throughput, 8-channel slide.



Touchscreen

The LUNA-FX7[™] has a 7-inch capacitive touchscreen for navigating the user interface.

Slide port

The automated slide port enables one-time slide insertion.

Power button

The power button is used for the main power control.

USB ports

USB ports allow the user to transfer or print cell count data. Data may be transferred via USB drive or the provided WiFi dongle. Counting data may be printed using the LUNA-FX7[™] Printer (P17001).

Ethernet port

The Ethernet port allows the instrument to be connected to a computer network. The CountWire[™] software package enables automated data synchronization and the ability to remotely operate the LUNA-FX7[™].

Power inlet

Connect the power inlet of the instrument to an electrical outlet with the supplied AC adapter and power cord.

LUNA-FX7™ Cell Counting Slides

The LUNA-FX7[™] gives you the flexibility to use various counting slide formats. The LUNA-FX7[™] is compatible with the LUNA[™] 1-, 3-, 8-Channel and Reusable Slide formats in addition to the standard LUNA[™] Cell Counting Slides and PhotonSlides[™]. With single-time slide insertion, the LUNA-FX7[™] is able to count all slide chambers at one time without needing

to remove and reinsert a slide. The increased counting volume yields more accurate and consistent results.

Channel No.	1 Channel	2 Channel	3 Channel	8 Channel	Reusable
	LUNA™ 1-Channel Slides	LUNA™ Cell Counting Slides / PhotonSlide™	LUNA™ 3-Channel Slides	LUNA™ 8-Channel Slides	LUNA™ Reusable slides
Compatible Slides		s.	A	in the second	
Sample Throughput	1 sample	Up to 2 samples	Up to 3 samples	Up to 8 samples	1 sample
Sample Loading Volume	50 µL	10 µL/chamber	10 µL/chamber	10 µL/chamber	10 µL/chamber
Analysis Volume	5.1 µL	1.3 µL/chamber	1.3 µL/chamber	0.5 µL/chamber	1.3 µL/chamber
0					
		13			

2. Getting Started

Installation

Installation	Place the	e LUNA-FX7™ on a clean, level and sturdy surface.
	0	Avoid vibrations from other devices.
	0	Do not install the instrument in a location that will expose the device to intense ultraviolet light.
	0	Allow at least 5 cm (2 inches) free space at the back of the instrument to prevent overheating of the instrument.
	0	Allow at least 10 cm (4 inches) free space at the right of the instrument to insert/eject a cell counting slide easily.
	Connect	the instrument to electrical outlets using the supplied power cord and AC adapter.
	0	Make sure the power cords are appropriate for your region.
	0	Always use power cord and AC adapter provided or approved by Logos Biosystems. If appropriate cord is not used, the electrical safety of the instrument cannot be guaranteed.
	Connect	the supplied WiFi dongle to a USB port.
	(Optiona	I) Connect a LUNA-FX™ Thermal Printer (P17001) to a USB port.
Setup		

Push the power button located below the touchscreen to turn on the instrument. After LUNA-FX7™ a short beep, the company logo will appear, followed by the home screen. Startup

	BRIGHT FIELD CELL COUNTING TOTAL CELL COUNTING CELL COUNTING & VIABILITY	
0		ብ
	The home screen has three menus:	

Brightfield Cell Counting

- Select Total cell counting mode or Cell counting & viability mode.
- Total cell counting mode is used to enumerate total cell numbers without staining ce

lls.

- Cell counting & viability mode is used to count cells and calculate the viability of cel ls stained with Trypan Blue Stain, 0.4% (T13001) or Erythrosin B Stain (L13002) or y east stained with Methylene blue Stain, 0.02% (L13004).

Fluorescence Cell Counting

- Select Cell lines & primary cells mode or Cell lines & primary cells, Advanced mod e.

- Cell lines & Primary cells mode is used to count cells and calculate the viability of cells stained with fluorescence dyes, Acridine Orange/Propidium Iodide Stain (F23001).

- Cell lines & Primary cell mode may also be used to count cells expressing GFP an d/or RFP.

- Cell lines & Primary cells, Advanced mode is used to count cells and calculate the viability of cells with an improved cell detection and cell de-clustering capabilities.

Quality Control

- Quality Control mode is only functional upon registration of Logos Biosystems brightfi eld or fluorescent validation slides.

- The Quality Control menu is used to monitor the accuracy and variability of the instrument.

- The validation slides contain pre-spotted patterns or pre-fixed beads with a known c oncentration and viability.

- Utilizing the Quality Control feature can provide daily, weekly, or monthly validation r esults that may be graphically displayed, and/or downloaded.

Screen Saver The screen backlight will automatically turn off after 10 minutes of inactivity. Touching the screen will reactivate the instrument.

3. Counting Cells

Sample Preparation

Sample Staining Brightfield cell counting

For Total and viability cell counting, prepare a cell suspension according to standard procedures. Mix the sample, 1:1, with Trypan Blue Stain, 0.4% (T13001) or Erythrosin B Stain (L13002) or Methylene blue Stain, 0.02% (L13004). Mix gently, but thoroughly to ensure a homogenous suspension.

For total cell counting, load the sample directly onto the slide without staining the sample.

Fluorescent cell counting

Prepare a cell suspension according to standard procedures. Mix the sample, 9:1 (cells: stain), with Acridine Orange/Propidium Iodide Cell Viability Kit (F23001). Mix gently, but thoroughly to ensure a homogenous suspension.

Sample Loading Load the appropriate volume for each slide chamber according to the table below:

LUNA™ 1-Channel Slides	LUNA™ Cell Counting Slides & PhotonSlide™	LUNA™ 3-Channel Slides	LUNA™ 8-Channel Slides**	LUNA™ Reusable Slides
50 µL	10 µL	10 µL	10 µL	10 µL

** The LUNA™ 8-Channel Slides are multi-channel pipette compatible.

For easy and accurate loading, hold the slides by their edges and pipette at a 45-60° angle to the slide. Take care not to overload or under-load the chamber.

Counting with the LUNA-FX7™

Slide Insertion &

Removal

Select appropriate counting mode and navigate to appropriate counting screen.

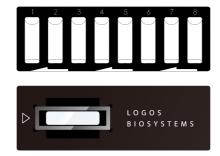
Press EJECT.

When inserting a slide into the instrument, ensure that the slide is facing up so that the arrow is showing on the right side and/or so that the lowest chamber designation is to the left.

Insert from this side.

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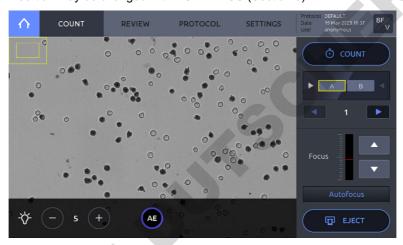


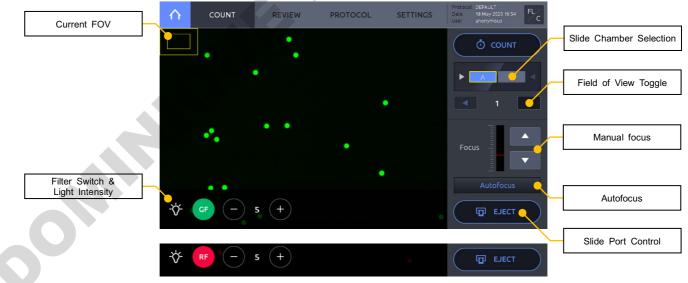


Press INSERT. The slide holder will automatically move into position.

To remove the slide, press **EJECT**. The slide holder will automatically extend out of the instrument and the slide may be removed.

# **Viewing Images** By default, upon slide insertion, the viewing light will automatically turn on and the LUNA-FX7[™] will perform an initial autofocus. Whether or not autofocus is performed upon slide insertion may be changed within **SETTINGS** (Section 8).





#### Light

By pressing the lamp icon in the bottom left corner of the screen, a light control panel will appear. The intensity can be adjusted as needed. Press AE to use the auto-exposure function. When in the fluorescence counting screen, the filters also may be switched between the BF, Green, and Red channels. Photobleaching will occur with procolonged exposure, so work appropriately.

Important ! Adjusting light intensity levels in the COUNT screen will only be applied to the live view mode. Exposure levels for brightfield cell counting are automatically adjusted. Exposure levels for fluorescence cell counting may only be adjusted within a protocol (Section 5).

#### Focusing

To bring cells into focus, press **Autofocus**. Focus may also be adjusted manually using the up & down arrows in the focus control bar.

#### Zoom

Zoom in or out by spreading or pinching two fingers. The outer box in the upper, left hand corner of the viewing window represents the current field of view. The inner box represents the view on the screen. Zooming in or out will cause the inner box size to decrease or increase.

#### Navigation

To view different slide chambers within a slide, select the chamber to be viewed by pressing a chamber on the slide image just under the **COUNT** button. To see different fields of view within a chamber, use the arrows located above the manual focus adjustment.

**Cell Counting** Prior to counting, confirm that the image is in focus for the first field of view. When the first field is in focus, press the **COUNT** button.

The LUNA-FX7[™] will count all slide chambers as designated in SETTINGS.

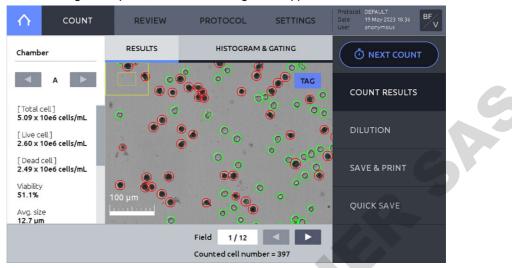
Counting time will vary depending on slide type, counting mode and the protocol used.

## Results

#### Results

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After counting is complete, the data and images will appear in the **RESULTS** window.



The counting results will be shown to the left of the screen.

Press the left or right arrows under **Chamber** to view the results and images for each counted chamber.

Press TAG to identify live (green circles) or dead (red circles) cells.

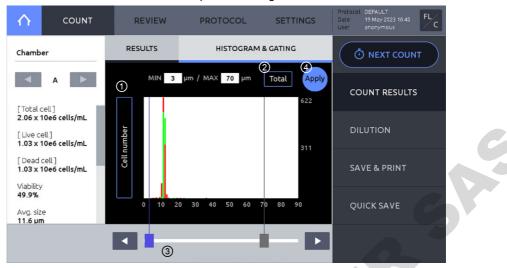


After fluorescence cell counting, BF, GF, and RF images can be viewed separately or in overlay.

#### **Histograms**

ON

#### Press HISTOGRAM & GATING to open the histogram window.



- Cell concentration or number can be graphed according to cell size.
   To toggle between cell concentration, cell cluster, and cell number press the Y-Axis title.
- ② Each histogram for total, live, and dead cells can be displayed. To switch between total, live, and dead, press the title box.
- ③ Cell size gating parameters may be changed by pressing the slider rectangles. An active slider will be highlighted in blue. Move the sliders by dragging or pressing the arrows.
- ④ Press Apply to set cell size gating parameters. Counting results will adjust accordingly.

		IEW	PROTO	COL	SETTINGS	Protocol DEFAULT Date 01 Dec 2021 15:47 User anonymous
	Pre-dilution factor 1: 10					
	Current Concentration 1.36 ×10e 6 Total	/mL	1	2	3	COUNT RESULTS
	Stock Concentration 1.36 ×10e 7	/mL	4	5	6	DILUTION
	Desired Concentration 1 ×10e 5 Final Volume 10 mL	/mL	7	8	9	SAVE & PRINT
•	CALCULATE		0		<b>←</b>	QUICK SAVE
	Dilute 0.074 mL of cell sample in 9.926	mL of buffe	ЭГ.			

#### Dilution Calculator Press DILUTION to open the Dilution Calculator.

The dilution calculator starts out with the concentration of total cells (live and dead) as the current concentration. The current concentration options are **Total**, **Live**, **Dead**, and **Custom**, allowing users to set the current concentration to be the total cell concentration, live cell concentration, dead cell concentration, or a custom cell concentration by pressing the blue box below the Current Concentration value.

If the cell counting was performed with the diluted sample from the stock cell solution, dilution instruction can be calculated from the stock cell concentration by entering pre-dilution factor. For example, enter "10" in the pre-dilution factor if the counted sample was 10-fold diluted

from stock solution. Enter "1" (default value) if the counted sample was not diluted from stock solution.

Enter the values for the desired concentration and final volume. Press **CALCULATE** and dilution instruction will appear in the grey message box.



#### Press SAVE & PRINT to open the save window.

Save

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Select the desired saving options:

Save Options	File Type	Description				
Analyzed image	TIF	Tagged images of cells				
Raw image	TIF	Untagged images of cells				
Report PDF		Report with data, images, and histograms				
Bioprocess	Onboard graph, CSV	Growth curve displayed onboard Growth rate, doubling time				

Press Path to select where files are to be stored.

Using the onscreen keyboard, provide the name and append the date and time by pressing the **+Date/Time** button.

Press **Save**. A folder name will be created with the name provided. The folder will contain subfolders matching each of the counted chambers, e.g. 'Ch A', or 'Ch 1'.

When saving, one of the following must be selected: Analyzed image, Raw image, or Report. It is not possible to save only the Bioprocess.

Even if you specify the path as a USB drive, the Bioprocess will be saved only in the internal memory.

Pé

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**Print** To print a text summary of the counting results, make sure a LUNA-FX7[™] Thermal Printer (P17001) is connected to the LUNA-FX7[™] and press **Print**.

The printer should be connected before powering on the instrument.

The printed report will contain the cell count results and protocol details.

Cell count report         Instrument : LUNA-FX7 Cell Counter         Serial number : LU7-00-00000         Software version : 0.0.0         Instrument name : LU7-00-00000         Film name : HL60         Date : 16 Apr 2017 14:35:37         Security : Off         User : anonymous         File name : NoTitle         Counting mode : Fluorescence cell counting         Cell lines & Primary cells         Instrument setting         Slide type : 2 channel slide         Counted chamber area : A, B         Autofocusing channel in FL : BF         Autofocus upon slide insertion : On         Last calibration : 10 Apr 2017 11:45         Calibration value : 24 / 36 / 58 / 100 / 74         Protocol         Protocol         Protocol name : DEFAULT         GF exposure level : 5         Cell size : 30         Max. cell size : 70         GF threshold level : 5         RF threshold level : 5         RF threshold level : 5         Dilution factor : 1.11         Size gating: 3 ~ 60 µm         Cell count results         [Total cell] : 1.04 x 10e6 cells / mL         [Dead cell] : 6.62 x 10e4 cells / mL         Viability: 93.6 %         Av	Instrument : LUNA-FX7 Cell Counter Serial number : LU7-00-00000 Software version : 0.0.0	
Serial number : LU7-00-00000 Software version : 0.0.0 Firmware version : 0.0.0 Instrument name : LU7-00-00000 File name : HL60 Date : 16 Apr 2017 14:35:37 Security : Off User : anonymous File name : NoTitle Counting mode : Fluorescence cell counting Cell lines & Primary cells Instrument setting Slide type : 2 channel slide Counted chamber area : A, B Autofocusing channel in FL : BF Autofocus gon slide insertion : On Last calibration : 10 Apr 2017 11:45 Calibration value : 24 / 36 / 58 / 100 / 74 Protocol Protocol name : DEFAULT GF exposure level : 5 Cell size calculation: BF Min. cell size : 3 Max. cell size : 70 GF threshold level : 5 RF threshold level : 5 RF threshold level : 5 RF threshold level : 5 Dilution factor : 1.11 Size gating: $3 ~ 60 \ \mu m$ Cell count results [Total cell] : 1.04 x 10e6 cells / mL [Live cell] : 9.73 x 10e5 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 \ \mu m Total cell number : 221 Live cell number : 235	Serial number : LU7-00-00000 Software version : 0.0.0	
Software version : 0.0.0 Firmware version : 0.0.0 Instrument name : LU7-00-00000 File name : HL60 Date : 16 Apr 2017 14:35:37 Security : Off User : anonymous File name : NoTitle Counting mode : Fluorescence cell counting Cell lines & Primary cells Instrument setting Slide type : 2 channel slide Counted chamber area : A, B Autofocusing channel in FL : BF Autofocus upon slide insertion : On Last calibration : 10 Apr 2017 11:45 Calibration value : 24 / 36 / 58 / 100 / 74 Protocol Protocol name : DEFAULT GF exposure level : 5 RF exposure level : 5 Cell size calculation: BF Min. cell size : 70 GF threshold level : 5 RF threshold level : 5 Dilution factor : 1.11 Size gating: 3 ~ 60 μm Cell count results [Total cell] : 1.04 x 10e6 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 μm Total cell number : 251 Live cell number : 251	Software version : 0.0.0	
Firmware version : 0.0.0 Instrument name : LU7-00-00000 File name : HL60 Date : 16 Apr 2017 14:35:37 Security : Off User : anonymous File name : NoTitle Counting mode : Fluorescence cell counting Cell lines & Primary cells Instrument setting Slide type : 2 channel slide Counted chamber area : A, B Autofocusing channel in FL : BF Autofocused counting : On Autofocus upon slide insertion : On Last calibration : 10 Apr 2017 11:45 Calibration : 10 Apr 2017 11:45 Calibration : 10 Apr 2017 11:45 Calibration slide : 5 RF exposure level : 5 RF exposure level : 5 RF exposure level : 5 RF threshold level : 5 RF threshold level : 5 RF threshold level : 5 RF threshold level : 5 Dilution factor : 1.11 Size gating: $3 \sim 60 \mu\text{m}$ Cell count results [Total cell] : 1.04 x 10e6 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 $\mu\text{m}$ Total cell number : 251 Live cell number : 251		
Instrument name : LU7-00-00000 File name : HL60 Date : 16 Apr 2017 14:35:37 Security : Off User : anonymous File name : NoTitle Counting mode : Fluorescence cell counting Cell lines & Primary cells Instrument setting Slide type : 2 channel slide Counted chamber area : A, B Autofocusing channel in FL : BF Autofocus upon slide insertion : On Last calibration : 10 Apr 2017 11:45 Calibration value : 24 / 36 / 58 / 100 / 74 Protocol Protocol name : DEFAULT GF exposure level : 5 RF exposure level : 5 Cell size calculation: BF Min. cell size : 70 GF threshold level : 5 RF threshold level : 5 Dilution factor : 1.11 Size gating: 3 ~ 60 μm Cell count results [Total cell] : 1.04 x 10e6 cells / mL [Live cell] : 9.73 x 10e5 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 μm Total cell number : 251 Live cell number : 251	Firmware version : 0 0 0	
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Date : 16 Apr 2017 14:35:37 Security : Off User : anonymous File name : NoTitle Counting mode : Fluorescence cell counting Cell lines & Primary cells Instrument setting Slide type : 2 channel slide Counted chamber area : A, B Autofocusing channel in FL : BF Autofocus upon slide insertion : On Last calibration : 10 Apr 2017 11:45 Calibration value : 24 / 36 / 58 / 100 / 74 Protocol Protocol name : DEFAULT GF exposure level : 5 RF exposure level : 5 Cell size calculation: BF Min. cell size : 3 Max. cell size : 70 GF threshold level : 5 RF threshold level : 5 RF threshold level : 5 Dilution factor : 1.11 Size gating: 3 ~ 60 μm Cell count results [Total cell] : 1.04 x 10e6 cells / mL [Live cell] : 9.73 x 10e5 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 μm Total cell number : 251 Live cell number : 251 Live cell number : 235		
Security : Off User : anonymous File name : NoTitle Counting mode : Fluorescence cell counting Cell lines & Primary cells Instrument setting Slide type : 2 channel slide Counted chamber area : A, B Autofocusing channel in FL : BF Autofocus upon slide insertion : On Last calibration : 10 Apr 2017 11:45 Calibration value : 24 / 36 / 58 / 100 / 74 Protocol Protocol name : DEFAULT GF exposure level : 5 RF exposure level : 5 Cell size calculation: BF Min. cell size : 70 GF threshold level : 5 RF threshold level : 5 Dilution factor : 1.11 Size gating: 3 ~ 60 μm Cell count results [Total cell] : 1.04 x 10e6 cells / mL [Live cell] : 9.73 x 10e5 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 μm Total cell number : 251 Live cell number : 251		
User : anonymous File name : NoTitle Counting mode : Fluorescence cell counting Cell lines & Primary cells Instrument setting Slide type : 2 channel slide Counted chamber area : A, B Autofocusing channel in FL : BF Autofocused counting : On Autofocus upon slide insertion : On Last calibration : 10 Apr 2017 11:45 Calibration value : 24 / 36 / 58 / 100 / 74 Protocol Protocol name : DEFAULT GF exposure level : 5 RF exposure level : 5 Cell size calculation: BF Min. cell size : 3 Max. cell size : 70 GF threshold level : 5 RF threshold level : 5 Dilution factor : 1.11 Size gating: 3 ~ 60 μm Cell count results [Total cell] : 1.04 x 10e6 cells / mL [Dead cell] : 0.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 μm Total cell number : 251 Live cell number : 235		
File name : NoTitle         Counting mode : Fluorescence cell counting Cell lines & Primary cells         Instrument setting         Slide type : 2 channel slide         Counted chamber area : A, B         Autofocusing channel in FL : BF         Autofocused counting : On         Autofocus upon slide insertion : On         Last calibration : 10 Apr 2017 11:45         Calibration value : 24 / 36 / 58 / 100 / 74         Protocol         Protocol name : DEFAULT         GF exposure level : 5         Cell size calculation: BF         Min. cell size : 70         GF threshold level : 5         Dilution factor : 1.11         Size gating: 3 ~ 60 µm         Cell count results         [Total cell] : 1.04 x 10e6 cells / mL         [Live cell] : 9.73 x 10e5 cells / mL         [Dead cell] : 6.62 x 10e4 cells / mL         Viability : 93.6 %         Avg. size : 16.5 µm         Total cell number : 251         Live cell number : 235		
Cell lines & Primary cells         Instrument setting         Slide type : 2 channel slide         Counted chamber area : A, B         Autofocusing channel in FL : BF         Autofocus upon slide insertion : On         Last calibration : 10 Apr 2017 11:45         Calibration value : 24 / 36 / 58 / 100 / 74         Protocol         Protocol         Protocol name : DEFAULT         GF exposure level : 5         Cell size calculation: BF         Min. cell size : 70         GF threshold level : 5         RF threshold level : 5         Dilution factor : 1.11         Size gating: 3 ~ 60 µm         Cell count results         [Total cell] : 1.04 x 10e6 cells / mL         [Live cell] : 9.73 x 10e5 cells / mL         [Dead cell] : 6.62 x 10e4 cells / mL         Viability : 93.6 %         Avg. size : 16.5 µm         Total cell number : 251         Live cell number : 235		
Cell lines & Primary cells         Instrument setting         Slide type : 2 channel slide         Counted chamber area : A, B         Autofocusing channel in FL : BF         Autofocus upon slide insertion : On         Last calibration : 10 Apr 2017 11:45         Calibration value : 24 / 36 / 58 / 100 / 74         Protocol         Protocol         Protocol name : DEFAULT         GF exposure level : 5         Cell size calculation: BF         Min. cell size : 70         GF threshold level : 5         RF threshold level : 5         Dilution factor : 1.11         Size gating: 3 ~ 60 µm         Cell count results         [Total cell] : 1.04 x 10e6 cells / mL         [Live cell] : 9.73 x 10e5 cells / mL         [Dead cell] : 6.62 x 10e4 cells / mL         Viability : 93.6 %         Avg. size : 16.5 µm         Total cell number : 251         Live cell number : 235	Counting mode : Fluorescence cell counting	
Slide type : 2 channel slide         Counted chamber area : A, B         Autofocusing channel in FL : BF         Autofocus upon slide insertion : On         Last calibration : 10 Apr 2017 11:45         Calibration value : 24 / 36 / 58 / 100 / 74         Protocol         Protocol name : DEFAULT         GF exposure level : 5         Cell size calculation: BF         Min. cell size : 3         Max. cell size : 70         GF threshold level : 5         RF threshold level : 5         Dilution factor : 1.11         Size gating: 3 ~ 60 μm         Cell count results         [Total cell] : 1.04 x 10e6 cells / mL         [Live cell] : 9.73 x 10e5 cells / mL         [Dead cell] : 6.62 x 10e4 cells / mL         Viability : 93.6 %         Avg. size : 16.5 μm         Total cell number : 251         Live cell number : 235	Cell lines & Primary cells	
Slide type : 2 channel slide         Counted chamber area : A, B         Autofocusing channel in FL : BF         Autofocus upon slide insertion : On         Last calibration : 10 Apr 2017 11:45         Calibration value : 24 / 36 / 58 / 100 / 74         Protocol         Protocol name : DEFAULT         GF exposure level : 5         Cell size calculation: BF         Min. cell size : 3         Max. cell size : 70         GF threshold level : 5         RF threshold level : 5         Dilution factor : 1.11         Size gating: 3 ~ 60 μm         Cell count results         [Total cell] : 1.04 x 10e6 cells / mL         [Live cell] : 9.73 x 10e5 cells / mL         [Dead cell] : 6.62 x 10e4 cells / mL         Viability : 93.6 %         Avg. size : 16.5 μm         Total cell number : 251         Live cell number : 235		ł
Counted chamber area : A, B Autofocusing channel in FL : BF Autofocus upon slide insertion : On Last calibration : 10 Apr 2017 11:45 Calibration value : 24 / 36 / 58 / 100 / 74 Protocol Protocol name : DEFAULT GF exposure level : 5 RF exposure level : 5 Cell size calculation: BF Min. cell size : 70 GF threshold level : 5 RF threshold level : 5 RF threshold level : 5 Dilution factor : 1.11 Size gating: 3 ~ 60 µm Cell count results [Total cell] : 1.04 x 10e6 cells / mL [Live cell] : 9.73 x 10e5 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 µm Total cell number : 251 Live cell number : 235		T
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Autofocused counting : On Autofocus upon slide insertion : On Last calibration : 10 Apr 2017 11:45 Calibration value : 24 / 36 / 58 / 100 / 74 <u>Protocol</u> Protocol name : DEFAULT GF exposure level : 5 RF exposure level : 5 Cell size calculation: BF Min. cell size : 3 Max. cell size : 70 GF threshold level : 5 Dilution factor : 1.11 Size gating: 3 ~ 60 μm <u>Cell count results</u> [Total cell] : 1.04 x 10e6 cells / mL [Live cell] : 9.73 x 10e5 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 μm Total cell number : 251 Live cell number : 235		
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Calibration value : 24 / 36 / 58 / 100 / 74  Protocol  Protocol name : DEFAULT GF exposure level : 5 RF exposure level : 5 Cell size calculation: BF Min. cell size : 70 GF threshold level : 5 RF threshold level : 5 Dilution factor : 1.11 Size gating: 3 ~ 60 µm  Cell count results [Total cell] : 1.04 x 10e6 cells / mL [Live cell] : 9.73 x 10e5 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 µm Total cell number : 251 Live cell number : 235		
Protocol         Protocol name : DEFAULT         GF exposure level : 5         RF exposure level : 5         Cell size calculation: BF         Min. cell size : 70         GF threshold level : 5         Dilution factor : 1.11         Size gating: 3 ~ 60 µm         Cell count results         [Total cell] : 1.04 x 10e6 cells / mL         [Live cell] : 9.73 x 10e5 cells / mL         [Dead cell] : 6.62 x 10e4 cells / mL         Viability : 93.6 %         Avg. size : 16.5 µm         Total cell number : 251         Live cell number : 235		
Protocol name : DEFAULT         GF exposure level : 5         RF exposure level : 5         Cell size calculation: BF         Min. cell size : 3         Max. cell size : 70         GF threshold level : 5         RF threshold level : 5         Dilution factor : 1.11         Size gating: 3 ~ 60 μm         Cell count results         [Total cell] : 1.04 x 10e6 cells / mL         [Live cell] : 9.73 x 10e5 cells / mL         [Dead cell] : 6.62 x 10e4 cells / mL         Viability : 93.6 %         Avg. size : 16.5 μm         Total cell number : 251         Live cell number : 235	Protocol	
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RF exposure level : 5 Cell size calculation: BF Min. cell size : 3 Max. cell size : 70 GF threshold level : 5 Dilution factor : 1.11 Size gating: 3 ~ 60 µm Cell count results [Total cell] : 1.04 x 10e6 cells / mL [Live cell] : 9.73 x 10e5 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 µm Total cell number : 251 Live cell number : 235		
Cell size calculation: BF Min. cell size : 3 Max. cell size : 70 GF threshold level : 5 RF threshold level : 5 Dilution factor : 1.11 Size gating: 3 ~ 60 μm Cell count results [Total cell] : 1.04 x 10e6 cells / mL [Live cell] : 9.73 x 10e5 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 μm Total cell number : 251 Live cell number : 235		
Max. cell size : 70 GF threshold level : 5 RF threshold level : 5 Dilution factor : 1.11 Size gating: 3 ~ 60 μm Cell count results [Total cell] : 1.04 x 10e6 cells / mL [Live cell] : 9.73 x 10e5 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 μm Total cell number : 251 Live cell number : 235		
GF threshold level : 5 RF threshold level : 5 Dilution factor : 1.11 Size gating: 3 ~ 60 µm <u>Cell count results</u> [Total cell] : 1.04 x 10e6 cells / mL [Live cell] : 9.73 x 10e5 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 µm Total cell number : 251 Live cell number : 235	Min. cell size : 3	
RF threshold level : 5 Dilution factor : 1.11 Size gating: 3 ~ 60 µm Cell count results [Total cell] : 1.04 x 10e6 cells / mL [Live cell] : 9.73 x 10e5 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 µm Total cell number : 251 Live cell number : 235	Max. cell size : 70	
Dilution factor : 1.11 Size gating: 3 ~ 60 µm Cell count results [Total cell] : 1.04 x 10e6 cells / mL [Live cell] : 9.73 x 10e5 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 µm Total cell number : 251 Live cell number : 235	GF threshold level : 5	
Size gating: 3 ~ 60 µm Cell count results [Total cell] : 1.04 x 10e6 cells / mL [Live cell] : 9.73 x 10e5 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 µm Total cell number : 251 Live cell number : 235	RF threshold level : 5	
Cell count results [Total cell] : 1.04 x 10e6 cells / mL [Live cell] : 9.73 x 10e5 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 µm Total cell number : 251 Live cell number : 235		
[Total cell] : 1.04 x 10e6 cells / mL [Live cell] : 9.73 x 10e5 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 µm Total cell number : 251 Live cell number : 235	Size gating: 3 ~ 60 µm	
[Total cell] : 1.04 x 10e6 cells / mL [Live cell] : 9.73 x 10e5 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 µm Total cell number : 251 Live cell number : 235		
[Total cell] : 1.04 x 10e6 cells / mL [Live cell] : 9.73 x 10e5 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 µm Total cell number : 251 Live cell number : 235	Cell count results	
[Live cell] : 9.73 x 10e5 cells / mL [Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 µm Total cell number : 251 Live cell number : 235		1
[Dead cell] : 6.62 x 10e4 cells / mL Viability : 93.6 % Avg. size : 16.5 µm Total cell number : 251 Live cell number : 235		
Viability : 93.6 % Avg. size : 16.5 µm Total cell number : 251 Live cell number : 235		
Avg. size : 16.5 µm Total cell number : 251 Live cell number : 235		
Total cell number : 251 Live cell number : 235		
Dead cell number : 16		
	Dead cell number : 16	

**Quick Save** 

Press **QUICK SAVE** to save results with a default name and suffix designation. The appended suffix may be a sequential number or the date/time.

Default Quick save preferences may be pre-set in SETTINGS: SAVE & REVIEW.

# 4. Review

## **Reviewing Data**

<b>Review Images</b>	Press <b>REVIEW</b> .				
		REVIEW	PROTOCOL SETTINGS	Protocol DEFAULT Date 01 Dec 2021 14:10 User anonymous	
Select storage	SD 205.9 GB	RESULTS	PROTOCOL	REVIEW RESULTS	PROTOCOL
	1/1	Counting mode	Cell counting & Viability		Export
Page navigation	■ SSD ■ FX7_2021120112210	Channel Total cell concentration	A 1.36 x 10e6 cells/mL	- EXPORT	
	Ch A	Live cell concentration Dead cell concentration	7.15 x 10e5 cells/mL 6.48 x 10e5 cells/mL	- ERASE ALL	Erase ALL
	🖿 Ch B	Viability Average cell size	52.4% 11.7 μm		
	<b>F</b> X7_2021120112211	Total cell number	803	PREVIOUS COUNT	
	<b>F</b> X7_2021120112212	Live cell number Dead cell number	421 382		
Image >	FX7_2021120112213 FX7_2021120112213			REVIEW BIOPROCESS	
	FX7_2021120112214	Images > Rea	nalyze		
	FX7_202112011222C	COPY	PASTE DELETE		

Select SSD or USB drive.

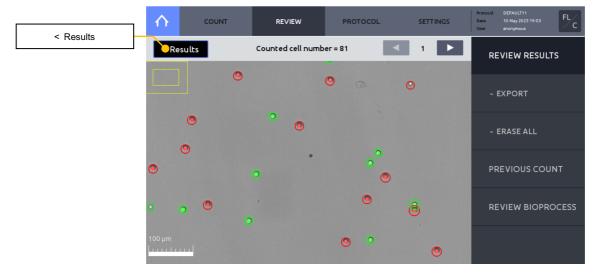
Navigate and open a folder from the internal or a USB drive. Select a subfolder, e.g., Ch A, Ch B. Cell counting results will appear. Max. 200 folders will be displayed per page. Press arrow button to navigate next page. Press page number to go to the specific page directly.

Insert a USB flash drive and press **EXPORT** to copy the counting data of the user in the current counting mode to the connected USB flash drive.

Press ERASE ALL to delete all counting data of the user in the current counting mode.

Press Images > to see images.

Press the **PROTOCOL** tab to check the protocol used.



Zoom in or out by using a pinching motion with two fingers.

Scroll through the captured images using the arrows.

To transfer files to a USB drive or delete files from the internal drive, press < **Results** to return to the main **Results** window. Use the command buttons at the bottom of the screen: COPY, PASTE, or DELETE.

#### **Reanalyze** Raw images may be reanalyzed using a different protocol.

Load or create the desired protocol.

! **Important** ! During reanalysis, changing exposure levels in the protocol will have no effect on the results of the reanalyzed counting data.

	REVIEW	PROTOCOL SETTINGS	Protocol DEFAULT Date 01 Dec 2021 14:10 User anonymous
<b>SSD v</b> 205.9 GB	RESULTS	PROTOCOL	REVIEW RESULTS
◀ 1/1 ►	Counting mode	Cell counting & Viability	
SSD 🔺	Channel	А	- EXPORT
- <b>F</b> X7_2021120112210	Total cell concentratio	,	
- Ch A	Dead cell concentratio	·····,····	- ERASE ALL
🖿 Ch B	Viability	52.4%	
<b>F</b> X7_2021120112211	Average cell size Total cell number	11.7 µm 803	PREVIOUS COUNT
EX7_2021120112212	Live cell number	421	
<b>F</b> X7_2021120112213	Dead cell number	382	
FX7_2021120112214	Images > F	eanalyze	REVIEW BIOPROCESS
<b>FX7_2021120112214</b>			
■ FX7_2021120112220 ▼		PASTE DELETE	

Press **REVIEW** and select a folder from the SSD(internal storage) or USB drive. Select the subfolder/chamber to be reanalyzed.

Press Reanalyze.

$  \land  $	COUNT	REVIEW	PROTO	DCOL	SETTINGS	Protocol DEFAULT Date 19 May 2023 11:19 User anonymous
User/File	Date/Time	[Total cell]	[Live cell]	[Dead cell]	Viability	REVIEW RESULTS
anonymous A	19 May 2023 10:43:20	3.73 x 10e6 2254	8.33 x 10e5 503	2.90 x 10e6 1751	22.3	REVIEW RESULTS
					51.1	
					98.5	PREVIOUS COUNT
anonymous FX7 00002 A					26.3	
					18.7	- EXPORT
					51.9	
					48.7	- ERASE ALL
		2.33 x 10e6 1407			48.8	
					27.5	REVIEW BIOPROCES
					38.8	
anonymous HL60 A	18 May 2023 14:37:27	2.33 x 10e6 1409		1.69 × 10e6 1021	27.5	<b>v</b>

#### **Previous Counts** Press **PREVIOUS COUNT** to see a list of previous counts.

A summarized version of each count that includes User/File, Date/Time, Total cell concentration, Live cell concentration, Dead cell concentration, Viability, Average size, and Protocol is automatically saved to the internal drive.

Live cell concentration, Dead cell concentration and Viability are not available In the Brightfield – Total cell counting mode.

Insert a USB drive and press EXPORT to save the data as a CSV file to the USB drive.

Press **ERASE ALL** to delete all stored counts. This will not delete reports or images of the corresponding count, if they were saved to the internal drive.

# 5. Protocols

# **Protocol Selection**

#### Default Protocol

Each counting mode comes with the following pre-set default counting protocols. These protocols cannot be edited.

Counting mode	Default Protocol				
Bright Field Cell Counting - Total Cell Counting	DEFAULT		YEAST		
Bright Field Cell Counting - Cell Counting & Viability	DEFAULT	IQOQ-BF	YEAST		
Fluorescence Cell Counting - Cell Lines & Primary Cells	DEFAULT	IQOQ-FL			
Fluorescence Cell Counting - Cell Lines & Primary Cells, Advanced	DEFAULT	IQOQ-FL-A	YEAST		

The **DEFAULT** protocols, by design, will provide optimal results for most cell types, but protocols for specific cell types or applications may need to be optimized.

The **IQOQ-BF** or **IQOQ-FL** or **IQOQ-FL-A** protocols, applied only for quality control and validation purposes.

The **YEAST** protocols, by design, will provide optimal results for most yeast types, but protocols for specific yeast types or applications may need to be optimized.

Customized protocols for specific cell types may be created. To create a new protocol, select any protocol and press **SAVE AS**.

Protocols

Creating

	REVIEW	N	PROTOCO	L	SE	ETTINGS	Protocol Date User	DEFAULT 01 Dec 2021 12:27 anonymous	BF
Protocol List  DEFAULT	Min. search size (1~89um)			redu	ise ction ~9)	Dilution factor (1~100)			
YEAST									
NEW PROTOCOL2	7	24	5	5	5	1			
	-		T				▼	•	▼
	LOA	٩D	ED			DELE	ΞTE	SAVE	AS

₼

Rename the protocol and press **Save**. The newly created protocol will appear in the list of protocols.

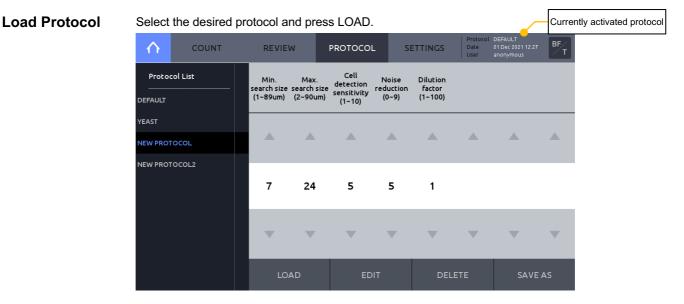
SAVE AS PRO	οτοςοι	-	DEVIE			οτος		CCT	TINCC		rotocol DE ate 21	FAULT Apr 2020 00-35	× T
P DEFA													
	NEW PF	ROTOCO	)L								×		
	1	2	3	4	5	6	7	8	9	0			
	Q	w	E	R	Т	Y	U	Ι	ο	Р			
	Α	S	D	F	G	Н	J	K	(   L	•			
	û	z	x	с	V	В	Ν	м	,	-	٢		
	Add	Date/T	ime			Spa	ace			Sa	ave		
			20	AU			ы		U			JAVE	AS

#### **Editing Protocols**

	COUNT	REVIEV	V	PROTOCO	L S	ETTINGS	Protocol Date User	DEFAULT 01 Dec 2021 12:27 anonymous	BF
Protoco  DEFAULT	l List	Min. search size (1~89um)		Cell detection sensitivity (1~10)	Noise reduction (0~9)	Dilution factor (1~100)			
YEAST									
				_	_	_		_	
NEW PROTO	NEW PROTOCOL2		24	5	5	1			
		-	•		▼	▼	•		•
		LOA	D	ED	лт	DEL	ETE	SAVE	۹S

Select a protocol that is not the *Default* protocol.

Press **EDIT**. This will activate the arrows for each parameter, turning them black. Press the arrows to adjust the values of each parameter. Press **SAVE AS** to change the protocol name. Press **LOAD** to save the edited protocol under the selected name and activate it.



The current active protocol name can be seen in the upper right hand corner of the screen.

## **Protocol Parameters**

**Brightfield Cell** 

Counting

**Parameters** 

BF DEFAULT 01 Dec 2021 12:28 COUNT REVIEW PROTOCOL SETTINGS  $\wedge$ Cell detection sensitivity (1~10) Protocol List Min. Max. search size search size (1~89um) (2~90um) Noise reduction (0~9) Dilution factor (1~100) 7 24 5 5 1 -V V V -

#### Parameters for [Brightfield cell counting-Total cell counting]

Parameter	Range	DEFAULT*		
Min. search size (µm)	1-89	7		
Max. search size (µm)	2-90	24		
Cell detection sensitivity	1-10	5		
Noise reduction	0-9	5		
Dilution factor	1-100	1		

#### Parameters for [Brightfield cell counting-Cell counting & Viability]

$\land$	COUNT	REVIE	N	PROTOCO	L S	ETTINGS		DEFAULT 01 Dec 2021 12:28 anonymous	BF
Protocol  DEFAULT	List	Min. search size (1~89um)			Live cell sensitivity (1~10)	Noise reduction (0~9)	Dilution factor (1~100)		
IQOQ-BF YEAST									
		7	24	5	5	5	2		
		•	•	•	•	▼	▼	•	•
		LO	٩D	ED	ЭΙТ	DEL	ETE.	SAVE	AS

Parameter	Range	DEFAULT*
Min. search size (µm)	1-89	7
Max. search size (µm)	2-90	24
Cell detection sensitivity	1-10	5
Live cell sensitivity	1-10	5
Noise reduction	0-9	5
Dilution factor	1-100	2

The IQOQ-BF on the Protocol List is the protocol that is used for IQOQ with the brightfiled validation slide. Protocol YEAST is an optimized protocol that is used for yeast cell counting.

Min./Max. search size	Search size refers to the approximate cell size that the algorithm recognizes as potential cell objects. By adjusting Min. and Max. search size, objects sized within the setting value will be listed as a potential cell candidate.						
	Clustered objects larger than the Max. search size will not be excluded from the search. Rather, the algorithm will utilize morphological information to identify individual objects within the cluster that are within the search size parameters. For the most accurate results, it is recommended to set the Min./Max. search size window as narrow as possible to encompass the expected cell size range.						
Cell detection sensitivity	Cell detection sensitivity refers to the sensitivity of object separation from the background. A higher Cell detection sensitivity value will increase detection of signals from weakly stained cells or smaller objects, but can also increase false positive calls.						
Live cell sensitivity	Live cells with intact cell membranes exclude Trypan blue, Erythrosin B and Methylene blue. The dyes stain the cytoplasm of dead cells with compromised membranes. As a result, object intensity of unstained live cells is brighter than the stained dead cells. A higher Live cell sensitivity will decrease the intensity cutoff value and increase the number of live cells detected.						
	Live Cell Sensitivity is not available in the protocol of the Total cell counting mode.						
Noise reduction	This option allows for the adjustment of background noise during counting. With more noise reduction, the instrument will not detect weakly stained objects. With lower noise reduction, the instrument can detect objects with fainter signals.						
Dilution factor	The dilution factor is used to calculate cell concentrations accurately. The default dilution factor is preset as 1 for Total cell counting and as 2 for Total cell & viability counting (assuming a 1:1 ratio of stain to cell suspension).						
	This value can be modified according to the dilution of the original sample in increments of 1 between 1-10 and, increments of 10 between 10 -100. For users handling highly dense cell						

between 1-10 and, increments of 10 between 10 -100. For users handling highly dense cell cultures. For highly dense cultures, serial dilutions and several counts with appropriately adjusted dilution factors may be necessary.

#### Fluorescence Cell Parameters for [Fluorescence cell counting-Cell lines & primary cells]

Counting Parameters – Cell lines & Primary cells mode

$\land$	COUNT	RE	VIEW	PROTOCO	DL	SETTINGS	Date User	12 Jul 2021 19:37 anonymous	FL'/C
Proto	col List	GF exposi leve (0.1~1	l level				GF threshold level (1~10)	RF threshold level (1~10)	Dilution factor (1~10)
IQOQ-FL									
		5	5	BF	3	70	5	5	1.11
				T	•	•	▼	▼	•
			LOAD	E	DIT	DE	LETE	SAVI	EAS

eter	Range	DEFAULT*

GF exposure level	0.1-10	5
RF exposure level	0.1-10	5
Cell size calculation	BF/FL	BF
Min. cell size	1-89	3
Max. cell size	2-90	70
GF threshold level	1-10	5
RF threshold level	1-10	5
Dilution factor	1-10	1.11

The IQOQ-FL on the Protocol List is the protocol that is used for IQOQ with the fluorescence validation slide.

Important ! The value of total concentration printed on the validation slide label, using the IQOQ-FL may differ from that of using the default protocol because the label value is determined with the IQOQ-FL protocol, which is for the purpose of IQOQ and Quality Control mode.

GF/RF ExposureThe exposure for each channel can be adjusted. Increase exposure if the preview image is dim<br/>and only a few cells are visible. Lower exposure if the preview image is too bright and<br/>background noise is high. Determine optimal exposure values empirically.

- Cell Size A mode between brightfield and fluorescence can be selected. The selected mode is used to measure the cell size.
  - If FL is selected, the cell size is measured based on the fluorescence signals so the results may change depending on the exposure level.

**Min./Max. cell size** Use these parameters to adjust the minimum and maximum cell sizes to be included in results. The base unit is 1 micrometer.

**GF/RF threshold level** Green and Red fluorescence threshold will determine the level of threshold during the image processing. Increasing the threshold will lead to fewer cells being detected by increasing the background level that is subtracted. Conversely, decreasing the threshold will lead to more cells being detected.

**Dilution Factor** The default dilution factor is pre-set as 1.11 for the standard Acridine Orange and Propidium iodide staining protocol, (e.g. 18 µL cells + 2 µL AO/PI.)

Assuming a final volume of 20  $\mu$ L, the dilution factor may be adjusted according to the table below:

Dilution factor	1	1.11	1.18	1.25	1.44	1.66	2	2.5	3.33	5	10
Sample volume	20 µL	18 µL	17 µL	16 µL	14 µL	12 µL	10 µL	8 µL	6 µL	4 µL	2 µL

Fluorescence Cell

Counting Parameters – Cell lines & Primary cells, Advanced mode Parameters for [Fluorescence cell counting-Cell lines & primary cells, Advanced]

$\land$	COUNT	REVIE	W	PROTOCO	L S	ETTINGS	Protocol Date User	DEFAULT 01 Dec 2021 14:1 anonymous	FL ⁷ A
Proto	col List	GF exposure level (0.1~10)	RF exposure level (0.1~10)	Min. search size (1~89um)		Declumping sensitivity (1~10)	Min. FL intensity (0~10)	Min. roundness (0~9)	Dilution factor (1~10)
IQOQ-FL-A YEAST	A								
		5	5	7	30	5	0	3	1.11
		-	•	▼	▼	▼	•	▼	•
		LO	AD	EC	лт	DELI	ETE	SAVI	EAS

Parameter	Range	DEFAULT*		
GF exposure level	0.1-10	5		
RF exposure level	0.1-10	5		
Min. search size (µm)	1-89	7		
Max. search size (µm)	2-90	30		
Declumping sensitivity	1-10	5		
Min. FL intensity	0-10	0		
Min. roundness	0-9	3		
Dilution factor	1-10	1.11		

The IQOQ-FL-A on the Protocol List is the protocol that is used for IQOQ with the fluorescence validation slide.

Important ! The value of total concentration printed on the validation slide label, using the IQOQ-FL-A may differ from that of using the default protocol because the label value is determined with the IQOQ-FL-A protocol, which is for the purpose of IQOQ and Quality Control mode.

Protocol YEAST is an optimized protocol that is used for yeast cell counting.

- GF/RF ExposureThe exposure for each channel can be adjusted. Increase exposure if the preview image is<br/>dim and only a few cells are visible. Lower exposure if the preview image is too bright and<br/>background noise is high. Determine optimal exposure values empirically.
- **Min./Max. search** Size Search size refers to the approximate cell size that the algorithm recognizes as potential cell objects. By adjusting Min. and Max. search size, objects sized within the setting value will be listed as a potential cell candidate.

Clustered objects larger than the Max. search size will not be excluded from the search. Rather, the algorithm will utilize morphological information to identify individual objects within the cluster that are within the search size parameters. For the most accurate results, it is recommended to set the Min./Max. search size window as narrow as possible to encompass the expected cell size range.

DeclumpingDeclumping sensitivity refers to the sensitivity of cell separation from the cluster of cells.<br/>Higher levels of declumping sensitivity will lead to the detection of more cells in a cluster, but<br/>may recognize internal cell structure as separate objects.

- **Min. FL intensity** Min FL intensity is used to set the minimum Green and Red fluorescence intensity values to be detected as cells. Objects with fluorescence intensity lower than Min. FL intensity value are excluded from counting. Increased Min. FL intensity value will remove objects with weak fluorescence intensity. Conversely, decreased Min FL. Intensity value will detect more objects with weak fluorescence intensity.
- **Min. roundness** Roundness is the measure of how closely the shape of a cell approaches mathematically perfect circle. Roundness is expressed as a value between 1 and 0, Roundness = 1 for a perfect circle and Roundness = 0 for a line segment. Higher value will lead to the counting of rounder cells and excludes objects with less roundness. Lower values are suitable for counting cells with irregular shapes.

**Dilution Factor** The default dilution factor is pre-set as 1.11 for the standard Acridine Orange and Propidium iodide staining protocol, (e.g. 18 µL cells + 2 µL AO/PI.)

Assuming a final volume of 20  $\mu\text{L},$  the dilution factor may be adjusted according to the table below:

Dilution factor	1	1.11	1.18	1.25	1.44	1.66	2	2.5	3.33	5	10
Sample volume	20 µL	18 µL	17 µL	16 µL	14 µL	12 µL	10 µL	8 µL	6 µL	4 µL	2 µL

# 6. Bioprocess Feature

## **Bioprocess Feature**

#### **Bioprocess**

The LUNA-FX7[™] bioprocess feature enables automated tracking of multiple bioprocessing activities. The bioprocess feature tracks individual batches according to protocol and will calculate and chart growth rates, doubling times, and viabilities based on count data.

$\mathbf{h}$	COUNT	REVIEW	PROTOCOL	SETTINGS	Protocol DEFAULT1 Date 03 Apr 2020 22:11 User anonymous		
Protocol List		RESULTS		GRAPH	REVIEW RESULTS		
DEFAULT2 DEFAULT3		Start Runs	24 Mar 2020 15:0 5	3:46	PREVIOUS COUNT		
DEFAULT4 DEFAULT5	_	– Last result ––– Date	27 Mar 2020 11:2	1:18	REVIEW BIOPROCESS		
HL60 K562	_	Elapsed Growth rate Doubling time Total cells	68.2922 hours 0.0360951 / hour 19.2034 hours		- EXPORT		
M1	v	Viability	4.87 × 10e6 cells , 88.4 %	/ mL	- ERASE ALL		

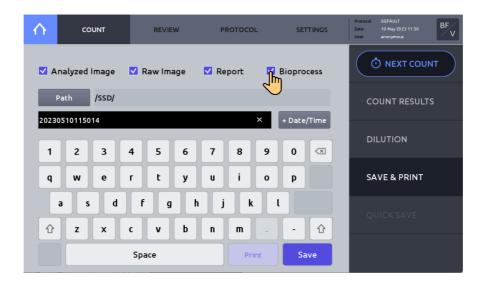
- **Creating a** 1. To create a new bioprocess, press **PROTOCOL** and create a new protocol.
- **Bioprocess** 2. Edit and save the new protocol as needed.

Protocol

Record Bioprocess1. To record bioprocess data, go to SETTINGS within a counting mode. Set Counting chamber<br/>area is set to 'Current'. If the Counting chamber area is not set to 'Current', bioprocessing<br/>data will not be saved.

2. Press **PROTOCOL** and load the appropriate protocol. When you record bioprocess data of the same cell, make sure that the same protocol you have used is loaded before you count.

- 3. Press the COUNT.
- 4. Press SAVE & PRINT.
- 5. Select either 'Analyzed Image' or 'Raw Image' or 'Report' AND select 'Bioprocess'.



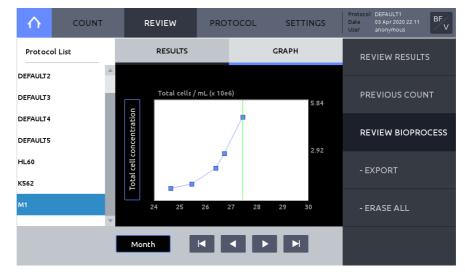
#### Review/Export 1. Select REVIEW.

#### Bioprocess Data 2. Press REVIEW BIOPROCES.

3. From the protocol list on the left, select the protocol used to create your bioprocess data.

4. Insert a USB drive and press **EXPORT** to save selected bioprocess data as a CSV file and graph image file to the USB drive.

5. Press **ERASE ALL** to delete the selected bioprocessing data. The data, but not the protocol will be deleted.



#### Press GRAPH to view charted results.

Press the Y-axis title box to alternate between 'Total cell concentration', 'Live cell concentration', and Viability'.

Press 'Day', 'Month' or 'Year' to alter X-axis scale.

# Graph

# 7. Quality Control

# **Quality Control Mode**

#### **Quality Control**

**Quality Control** mode is used to monitor the performance of the LUNA-FX7[™]. The Quality Control features may only be used in conjunction with the Logos Biosystems fluorescence or brightfield validation slides. Validation slides contain a pre-spotted pattern (brightfield) or pre-fixed beads (fluorescence) of known concentration and viability.

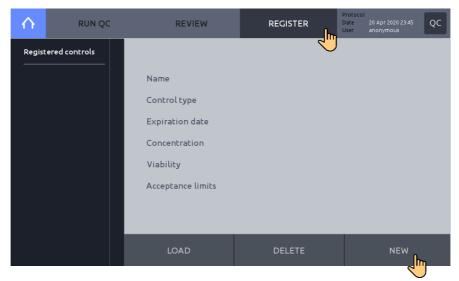


# Validation Slide Registration

Prior to use, validations slides must be registered.

Cell Counter Validation Slide-FL	Cell Counter Validation Slide-BF
Cat # L72030 1.15 x 10e8 /mL, 50.2%	Cat # L72040 4.4 x 10e6 /mL, 50%

Press Quality Control, press REGISTER.



Press NEW.

× > Name FL VALIDATION Name > Product key > Assay value > Acceptance limits 1 2 3 4 5 6 7 8 9 0 × т Υ U о Ρ Q w Е R Т F κ Α S D G н J L z х с v В Ν м Space

Enter a Name, then press the right arrow button [>] to move to 'Product key'.

Enter the 20 digit Product key. Press the 'space' icon to advance. Press OK.

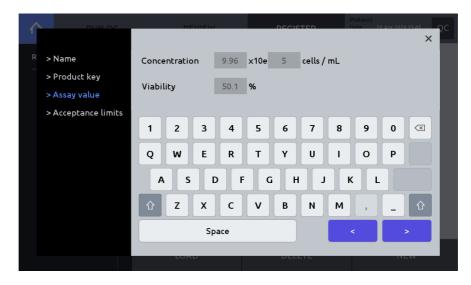
Confirm control type: Fluorescence or Brightfield and Expiration date.

- ! Important ! The product key is included with the product information of the validation slide. Contact <a href="mailto:sales@logosbio.com">sales@logosbio.com</a> if the product key is lost or missing.
- ! Important ! The Validation slide-FL have a limited lifetime and the reliability of a validation slide can no longer be guaranteed. Therefore, to ensure the accuracy and reliability of the QC data, it is recommended that replacement validation slides are purchased prior their stated expiration.



Press the right arrow button [>] to move to 'Assay value'.

Confirm that the Assay value is correct. If the Assay value differs from what was provided with the validation slide, check to ensure the product key was entered correctly. If entered, correctly, contact <u>sales@logosbio.com</u>



Set Acceptance limits (%). Acceptance limits produce upper and lower boundaries in QC graphing. Press **Save** to complete registration.

PUNOC			EVIEW			DECK	TED		Protocol Date 2	0 Apr 202	0.23:47	ρc
											×	
> Name	Conce	entrati	on		±	10	%					
> Product key												
>Assay value	Viabi	lity			±	10	%					
> Acceptance limits												
	1	2	3	4	5	6	7	8	9	0		
	Q	w	E	R	т	Y	U	I	ο	Р		
	A			• F		i   F	L L	K	(   L	.][		
	Û	z	x	С	V	В	N	м	,	_	Û	
			Spa	ace					<	Sa	ave	
		LOF				DLL						

After completing registration, the validation slide information may be viewed by selecting the appropriate registered control in the **REGISTER** main page, and pressing **Load**.

$  \land  $	RUN QC	REVIEW	REGISTER	Protocol Date 20 Apr 2020 23:47 User anonymous
Registered	controls			
<b>FL VALIDATION</b>		Name	FL VALIDATION	
		Control type	Fluorescence	
		Expiration date	31 Dec 2020	
		Concentration	9.96 $ imes$ 10e5 cells / mL	
		Viability	50.1 %	
		Acceptance limits	± 10 % / ± 10 %	
		LOAD	DELETE	NEW

## Performing Quality

Control

**y** Navigate to **Quality Control** mode and press **REGISTER.** Select a validation slide from the list of Registered controls. Press **LOAD**.

Press **RUN QC** in the main **Quality Control** screen, insert the validation slide, and press the **COUNT** button.



After counting, the QC graph will appear. Confirm that the measured value is within the acceptance limits established during slide registration.

Press RESULTS to see the counting data.

If the results are not within the acceptance range, redo RUN QC steps.

If not met again, contact your local distributor or Logos Biosystems.

$\land$	RUN QC		REVIEW	REGISTER	Protocol FL VALIDATION Date 20 Apr 2020 23:49 User anonymous
	GRAPH		F	RESULTS	
R	mits ± uns 1 ast result	10 % / ± 10 %			Rame Q FL VALIDATION Control type
	Date Concentration Viability	20 Apr 2020 2 1.04 x 10e6 ce 51.6 %			Fluorescence Expiration date 31 Dec 2020 Concentration
C	Result	ОК	<b></b>		9.96 x 10e5 cells / mL Viability 50.1 %
	Expiration Concentration Viability	31 Dec 2020 9.96 x 10e5 ce 50.1 %	ells / mL		Acceptance limits ± 10 % /± 10 %

To re-run QC, press NEXT COUNT, then press the COUNT button.

#### Review Press REVIEW.

Select a validation slide from the File list on the left.

$\mathbf{h}$	RUN QC		REVIEW	REGIST		BF VALIDATION 17 Aug 2020 11:56 anonymous	QC
File lis	t		GRAPH		RES	SULTS	
BF VALIDA	ATION		Total /mL (x 10e6)				
FL VALIDA	ITION	Concentration		10111213141516	17 18 19 20 21 22 23 24	4 25 26 27 28 29 30 3 EXPORT	8.80 4.40

Press **GRAPH** to view a graphical representation. Press the Y-axis title box to switch between 'Concentration' and 'Viability'. Press 'Day', 'Month' or 'Year' to alter X-axis scale.

Press **EXPORT** to export a CSV file with count data and graph images to a USB drive. Press **RESULTS** to view the most recent QC count.

R	UN QC	REVIEW	REGI			QC
File list		GRAPH		F	RESULTS	
BF VALIDATION		Limits ±109 Runs 9 Last result	% / ±10%			
		Concentration 4 Viability 4	7 Aug 2020 1 50 x 10e6 /m 9.8% K			
		Concentration 4	o Exp. date 40 x 10e6 /m 0.0%	۱L		

- ! **Important** ! The value of total concentration printed on the slide label may differ from the counting result of using the default protocol because the label value is determined with a different protocol, which is for the purpose of IQOQ and Quality Control mode.
- Important ! After the Quality Control counting is done, remove the validation slide from the device right away and store it in the case provided. The fluorescence prefixed beads may be photobleaching if they are exposed to light.

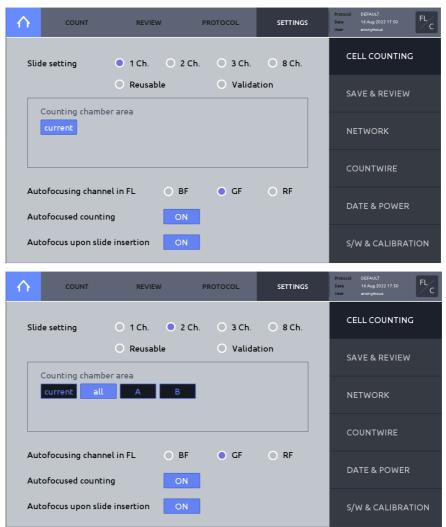
# 8. Settings

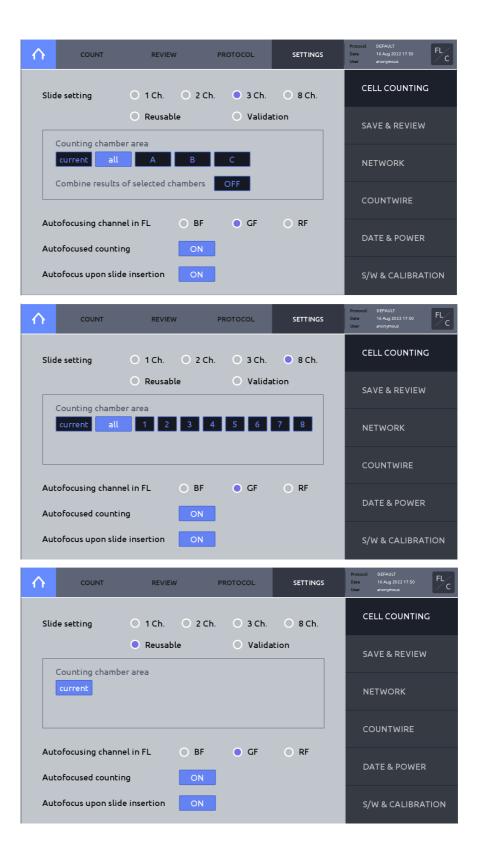
## LUNA-FX7[™] Settings

Screen Settings Navigate to either Brightfield or Fluorescence counting window and press SETTINGS. Within SETTINGS, software updates, date and time changes, and background calibrations may be performed. Additionally, options for 'Cell Counting', 'Save & Review', 'Network', 'CountWire' (only with CountWire™ package) and options may be adjusted.

**Cell Counting** 

Choose appropriate slide format and the chamber(s) to be counted.





$\mathbf{\wedge}$	COUNT	REVIE	W P	ROTOCOL	SETTINGS	Protocol Date User	DEFAULT 16 Aug 2022 17:50 anonymous	FL [/] /C
Slic	le setting	🔵 1 Ch.	🔵 2 Ch.	🔵 3 Ch.	🔘 8 Ch.	CE	ELL COUNTING	5
	Counting chambe	Reusat	ole	🔍 Valida	tion	SA	AVE & REVIEW	
		er area				NE	ETWORK	
						co	OUNTWIRE	
	tofocusing channel in FL tofocused counting		O BF	• GF	O RF	D#	ATE & POWER	
Aul	cofocus upon slid	e insertion	ON			s/	W & CALIBRAT	TION

#### Slide setting

Select a slide format and Counting chamber area option.

Counting chamber area:

o current

Counts the chamber that is being viewed in the live view of the Count screen.

o all

All chambers are counted.

• Chamber designation

One or more chambers may be selected.

• Combine results of selected chambers

This option can only be selected when using a 3Ch Slide. When the "Combine results of selected chambers" is activated, the Counting chamber area is automatically set to "all". The Mean and CV values of Chambers A, B, and C are calculated. Following A, B, and C in the Counting Result, "ALL" is added. When you save, the PDF and CSV files are stored in the "ALL" folder.

#### Autofocusing channel in FL

Select an autofocusing channel.

Option	Description
BF	Autofocusing is proceeded in the BF channel.
GF	Autofocusing is proceeded in the GF channel.
RF	Autofocusing is proceeded in the RF channel.

Autofocusing in the fluorescent channel is more efficient when the concentration of the sample is too low and the cell boundaries are faint.

This option is only available for fluorescence cell counting.

#### Autofocused counting

When Autofocused counting is activated, LUNA-FX7[™] will readjust the focus for each field of view during image capture (recommended to keep active).

#### Autofocus upon slide insertion

When Autofocus upon slide insertion is activated, autofocus is automatically performed when a slide is inserted.

#### Save & Review

#### Press Save & review on the right menu.

$\land$	COUN	IT	REVIEW	PROTOCOL	SETTING	s	Protocol Date User	DEFAULT 07 Dec 2021 14:42 anonymous	BF
Aut	o save	OFF					CE	LL COUNTING	
	ick & Auto s	save rule FX7					SA	VE & REVIEW	
	Suffix	Sequent	ce 1	O Date/Time	,		NE	TWORK	
	Next name	FX7_00001					со	UNTWIRE	
	stomized re						DA	TE & POWER	
		em logo in r cels, PNG forma		s/v	V & CALIBRAT	ION			

#### Auto save

When Auto save is activated, cell counting results are automatically saved according to the Quick & Auto save rule.

#### Quick & Auto save rule

o Name

This name will serve as the prefix for all saved counts.

o Suffix

Select **Sequence** to automatically add sequential numbers to the prefix name; OR, select **Date/Time** to automatically append date and time to the prefix name.

• Next name

Displays file name to be used for the next count to be saved.

#### Scale bar

Includes or excludes scale bar for Tag (Analyzed) images.

#### **Customized reports**

Allows PDF reports to be customized with preferred logo or image. Required image format: 160 x 160 pixels and PNG format.

#### Network

The LUNA-FX7[™] may be connected to a local network via Ethernet cable or WiFi.

Within SETTINGS, press NETWORK.

$\land$	COUNT	REVIEW	PROTOCOL	SETTINGS	Protocol Date User	DEFAULT 16 Aug 2022 17:51 anonymous	FL [°] /C
	Connect the LAN or inse	NA FX7	CI	ELL COUNTINC	5		
Net	work WiFi	<b>1</b> 92.16			SA	AVE & REVIEW	
٠	MAC ac Obtain an IP address	ddress : B4-2E-99-: automatically	3C-69-F9		N	ETWORK	
	Use the following IP a				co	OUNTWIRE	
	IP address: Subnet mask:		168         0         10           255         255         0		D	ATE & POWER	
	Default gateway	192	168 0 1		s/	W & CALIBRAT	TION

#### Ethernet connection

Connect an Ethernet cable to the instrument.

When connected, an IP address will appear on the screen in blue color.

#### WiFi connection

Insert the supplied WiFi dongle to a LUNA-FX7™ USB port.

#### Press WiFi.

Select appropriate WiFi, then press OK. Enter password, if necessary.

When the instrument is connected, an IP address will appear on the screen in blue color.

$\land$	COUNT	REVIEW	PROTOCOL	SETTINGS	Protocol DEFAULT Date 17 Aug 2022 13:57 User anonymous
		cable to the port o ert the WiFi dongle	n the back of the LU into a USB port.	NA FX7	CELL COUNTING
Net	twork WiFi		26.60.50		SAVE & REVIEW
•	Obtain an IP address	ddress : B4-2E-99 automatically	-3C-69-F9		NETWORK
	Use the following IP a				COUNTWIRE
	IP address: Subnet mask:	192 255	168         0         10           255         255         0		DATE & POWER
	Default gateway	192	168 0 1		S/W & CALIBRATION

$\land$	COUNT	REVIEW	PROTOCOL	SETTINGS		AULT Aug 2022 14:04 inymous	BF
	Connect the LAN or in	cable to the port o	n the back of the LUI			COUNTING	
Net	work Wil	🙃 Aligned	uest			& REVIEW	
	MAC Obtain an IP addre:	Rligned-g			NETW	ORK	
	Use the following II				COUN	ITWIRE	
	IP address: Subnet mask:	200	K Ca	ancel	DATE	& POWER	
	Default gateway	192	168 0 1		s/w &		ON

#### MAC Address

Available as a network address when you access the LUNA-FX7™.

- When using a Wi-Fi network with Mac address filtering
- When assigning a static IP

### **CountWire** This setting is required to use the CountWire[™] system.

### Press COUNTWIRE.

$\land$	COUNT	REVIEW PROTO		TOCOL	SETTI	NGS	Protocol Date User	DEFAULT 10 May 2023 18:32 anonymous	FL ² /C		
Cou	CountWire		ON						CE	LL COUNTING	i
Inst	rument name	LU7-0	5-0019	)4		Set	default		SA	VE & REVIEW	
Sto	rage IP address	192	168	0	84				NE	TWORK	
Sto	rage port	22							сс	OUNTWIRE	
Sta	tus	Conne	ected			A	pply		DA	TE & POWER	
									s/\	W & CALIBRAT	ION

#### CountWire

It must be ON to utilize the LUNA-FX7[™] as a part of the CountWire[™] system.

! Important ! If CountWire is on, data transfer via network by FTP does not work.

#### Instrument name

Required to distinguish the instruments in the CountWire[™] system.

The default instrument name is the serial number, but the name may be changed.

Press Set default to initialize the instrument name to the serial number.

#### Storage IP address

IP address of the CountWire[™] Data Storage. The same address that you input on the CountWire[™] Client. Ask network administrator for details.

#### Storage port

Port number of the CountWire™ Data Storage. The Storages port number is 22.

Press the **Apply** button after entering the required information. If it is successfully connected, you can see the status "Connected".

For more details for the CountWire[™], please refer to the CountWire[™] User Manual.

#### Date & Power Date & Time

The LUNA-FX7[™] uses a 24-hour clock that is preset to Korean Standard Time. Adjust the settings to the local date and time.

#### Press DATE & POWER.

Press Set Date & Time. Input the desired values. Press APPLY to save changes.

$\land$	COUNT	REVIEW	PROTOCOL	SETTINGS	Protocol DEFAULT Date 01 Dec 2021 15:20 User anonymous
	Date & Time		Set D	Pate & Time	CELL COUNTING
	Date Time	01 Dec 2021 15:20			SAVE & REVIEW
	Power saver	ON			NETWORK
	Turn off the instru	ument after 8	hours (1 ~ 24 ho	Jrs)	COUNTWIRE
					DATE & POWER
					S/W & CALIBRATION

#### Power saver

LUNA-FX7TM provides a **Power saver** to save energy and protect environment. Activate Power saver to automatically shut down the LUNA-FX7TM.

#### S/W & Calibration Software

Logos Biosystems continually provides software updates to ensure optimal performance. The current software version is displayed in **SETTINGS: S/W & CALIBRATION**.

1. The most recent version may be downloaded from the Logos Biosystems website (www.logosbio.com) into the root directory of a compatible USB drive.

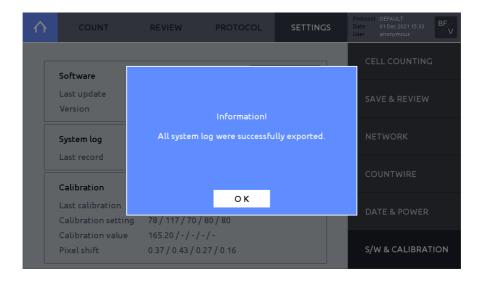
- 2. Press Software in the SETTINGS screen.
- 3. Insert the USB drive with the downloaded file and authentication key into a USB port.
- 4. Press Software update.
- 5. Press Start. If a software update has been found, press OK.
- 6. Press Restart, then the instrument will automatically shut down and then restart.

7. Prior to the next count, perform calibration.

$\wedge$	COUNT	REVIEW	PROTOCOL	SETTINGS	Protocol Date User	DEFAULT 10 May 2023 12:10 anonymous	BF
	Software		Softw	vare update	CE	ELL COUNTING	
	Last update Version	09 May 2023 14: 1.8.8 / 1.0.0	08		SA	VE & REVIEW	
	System log			Export	NE	ETWORK	
	Last record Calibration		Start	calibration	СС	DUNTWIRE	
	Last calibration Calibration setting	04 May 2023 09: 41 / 61 / 70 / 132			DA	ATE & POWER	
	Calibration value Pixel shift	166.13 / 0.00 / 0. -0.59 / -1.38 / -0.			s/	W & CALIBRAT	ION

#### System log

The LUNA-FX7[™] records the system log for quick diagnosis and service of the instrument. Recorded system log file can be exported to a USB drive. Submit the exported system log file to the authorized distributors or sales representatives for a faster service of an abnormal instrument.



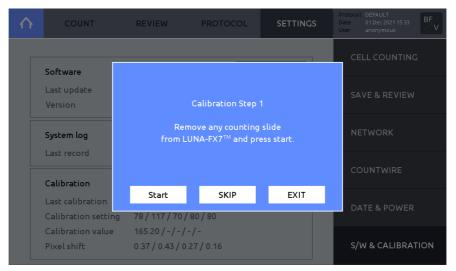
#### Calibration

The LUNA-FX7[™] is calibrated prior to shipping. Calibration only needs to be performed 1) after any software/firmware updates, and 2) after switching Trypan blue or Erythrosin B or Methylene blue brands or formulations.

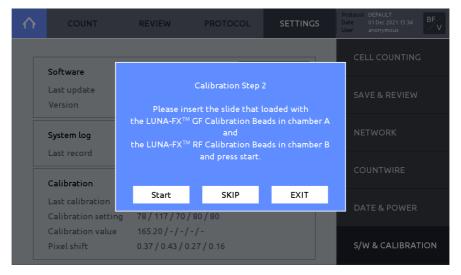
To perform calibration:

1. Press S/W & CALIBRATION.

2. Press Start calibration. The Calibration Step 1 window will appear.



- 3. Remove any counting slide from slide port.
- 4. Press START.
- 5. Press START or press SKIP if only fluorescence calibration is needed.
- 6. The Calibration Step 2 window will appear upon completion.



7. Load 10  $\mu$ L of the LUNA-FXTM GF Calibration Beads into chamber A and 10  $\mu$ L of the LUNA-FXTM RF Calibration Beads into chamber B of a new PhotonSlideTM. Vortex the calibration beads solution thoroughly before loading into the PhotonSlideTM.

- 8. Wait for 30 seconds for the beads to settle down.
- 9. Insert the slide face up and chamber A first into the slide port.

10. Press START; or, press SKIP, if fluorescence calibration is not needed.

11. Upon completion, the calibration value(s) and calibration date will be updated.

# 9. Data Transfer via Network

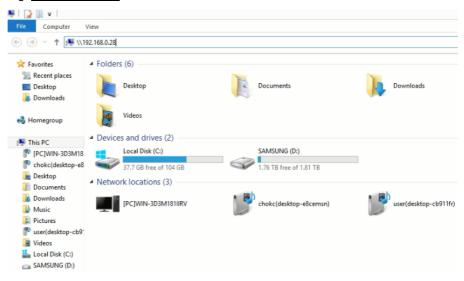
## **Network sharing**

Connected to User PC Connect the LUNA-FX7[™] to a network. Make note of the LUNA-FX7[™] IP address in the SETTINGS: NETWORK screen.

In your Windows PC, open File Explorer (Windows key + E)

Type the IP address connected to the LUNA-FX7™ in the location directory and press Enter.

#### e.g. <u>₩₩192.168.0.28</u>



You can double-click and open the luna-fx7 folder.

🖳   💽 🕕 = I	
File Home Sha	re View
€ ∋ - ↑ 📭 •	Network > 192.168.0.28
☆ Favorites [™] Recent places ■ Desktop [™] Downloads	luna-fx7
This PC (COMPARENT OF COMPARENT OF COMPAREN	
👊 Network	

The data in the folder is not stored in the PC. You may move data from the luna-fx7 folder to another drive in the PC.

If it is the initial access, it may require a log-in with User name and Password.

- o User name: logosbio
- Password: logosbio

	Windows Security ork credentials dentials to connect to: 192.168.0.33
P	User name Password Domain: LOGOSBIOSYSTEMS Remember my credentials
🐼 Acc	ess is denied.
	OK Cancel

You can right-click the folder to map the network drive or create shortcut for your convenience.

) 🤿 💌 ↑ 📑 ト Network	▶ 192.168.0.28
<ul> <li>★ Favorites</li> <li>★ Recent places</li> <li>➡ Desktop</li> <li>↓ Downloads</li> <li>♥ This PC</li> <li>♥ [PC]WIN-3D3M18⁻</li> <li>↓ Desktop</li> <li>↓ Documents</li> </ul>	Juna-fx7         Open         Open in new window         Add with ALZip         Compress using administrator authority         Compress to "192.168.0.zip"         Restore previous versions         Bitcasa
<ul> <li>Downloads</li> <li>Music</li> <li>Pictures</li> </ul>	Foxit Reader에서 PDF로 변환 Pin to Start
Videos	Map network drive
Local Disk (C:)	Сору
G SAMSUNG (D:)	Create shortcut
📬 Network	Properties

! Important ! If you cannot access the folder with the message below,



- 1) Please check if the IP address is correct and the LUNA-FX7[™] is well connected to the internet.
- If there is no connection issue, contact Logos Biosystems or your local distributor to receive a script in a Zip file to clean Windows authentication caches.
  - Unzip the received file and run the command file.
  - Type the IP address and press the Enter key.

e.g. <u>₩₩192.168.0.28</u>

- Press any key.
- Try to go through from the beginning.



# **10. Maintenance and Troubleshooting**

## Maintenance

**Powering on/off** To turn on the LUNA-FX7[™], push the power button below the touchscreen for at least a second.

To turn off the LUNA-FX7[™], press the power icon in the menu bar or push the power button for at least three seconds. Turn off the LUNA-FX7[™] at the end of each day.

#### Cleaning Safety

Turn the LUNA-FX7[™] off and disconnect the power cable before cleaning. Make sure that liquids do not enter any part of the instrument during cleaning. Do not use abrasi ve cloths or bleach solutions as this can cause topical damage.

#### Surfaces

Clean the surfaces of the instrument with a soft cloth dampened with distilled water. Wipe dry immediately. Do not pour or spray liquids directly onto the instrument. Do n ot wet electrical wires or connections in order to avoid electrical shock or damage.

#### Touchscreen

Clean the touchscreen with a soft cloth lightly dampened with an authorized LCD clean nsing detergent. Wipe dry immediately. Do not exert excessive force or pressure as the is can damage the touchscreen.

## Troubleshooting

Inaccurate Cell	Clumped cells
Count	Gently but thoroughly pipette your cell suspension to break up aggregates prior to counting.
	Too few or too many cells
	Cell concentrations of 5 x $10^4$ to 2 x $10^7$ cells/mL are optimal for counting.
	Dilute or concentrate cell suspensions accordingly.
	Fluorescence signal too strong or weak
	Adjust exposure level.
	Visible cells uncounted
	Adjust the protocol's detection sensitivity.
	Poorly focused images
	Make sure the first field is in focus before starting the count. This serves as a refere nce for the autofocus function.
	Improper slide insertion
	Make sure that the slide has been pushed completely to the end of the slide port.
	Improper sample loading
	Do not over- or under-fill the slide chambers.
	Optical components malfunctioning
	51

Optical components may be dirty or damaged. Please contact your local distributor or Logos Biosystems. Damaged or contaminated slide Use a new slide if it is disposable. Make sure that the counting area of the slide is transparent before loading the sampl e. Wear gloves and handle by the edges to avoid smudging and contamination. Incorrect dilution factor Adjust the dilution factor in the selected protocol or create a new protocol. Make sure the appropriate dilution factor has been selected. Slide Insertion Not complete close of the slide port If the 8-channel slide is selected in SETTINGS: CELL COUNTING, but other types of slides are inserted, the slide port is not completely closed. Select the right slide type in SETTINGS: CELL COUNTING, and insert the slide. **Data Transfer** Incompatible USB drive and Saving Some USB devices are undetectable or incompatible. Use the USB supplied with the i nstrument or use a USB 2.0. Failed wireless connection Check that the WiFi dongle is connected to the LUNA-FX7[™]. Check that the LUNA-F X7[™] is connected to a wireless network. Check that PC is connected to the same wir eless network as the LUNA-FX7[™]. Check your wireless network connection. **Failed Ethernet connection** Ensure the Ethernet cable is connected to the LUNA-FX7™ and restart the LUNA-FX7 тм Software Update Freezing during background calibration If calibration takes more than 10 minutes, reset the system by turning the power off a Errors nd then on. If calibration fails repeatedly, contact your local distributor or Logos Biosy stems. More than one software version on the USB drive Delete previous versions of software from the USB drive before downloading new soft ware. Incompatible USB drive Some USB devices are undetectable or incompatible. Use the USB drive supplied wit h the instrument or use a USB 2.0. Incorrectly saved or damaged software Download the file again into the root directory of the USB drive. Insert the USB drive and press Software update in the SETTINGS: SOFTWARE. If the problem persists, c ontact your local distributor or Logos Biosystems.

# **11. Product Specifications**

## LUNA-FX7[™] Automated Cell Counter

Physical and Technical Characteristics

	LUNA-FX7™ Basic Package	LUNA-FX7™ Advanced Package	
Onboard storage	250 GB	1 TB	
Additional software	-	Bioprocess software	
Cell size range	1 - 90 µ	m	
Detection range	1 x 10 ⁴ - 5 x 10 (Optimal: 5 x 10 ⁴ - 1		
Cell detection method	Automated brightfield & fluc	prescence microscopy	
Slide options	1-Ch, 2-Ch, 3-Ch, 8	-Ch, Reusable	
Measuring volume per chamber	0.5 - 5.1 μL/c (Each slide has different		
Optics	Brightfield, Dual f	luorescence	
Green fluorescence	Ex 470/40 nm, En	n 530/50 nm	
Red fluorescence	Ex 530/40 nm, Em 620/60 nm		
Focusing	Autofocus with manual focus option		
Instrument type	Benchtop cell counter		
Display	7-inch TFT LCD multi-touch screen, 1024 x 600 pixels		
Data format	PDF, CSV, TIFF		
Data export	USB, WiFi, E	thernet	
Printer	External printer	(optional)	
21 CFR Part 11	CountWire™ syste	em (optional)	
User management	CountWire™ syste	em (optional)	
IQ/OQ	Yes (option	onal)	
Dimensions	245 x 280 x 240 mm (9.6	6 x 11.0 x 9.4 inch)	
Weight	5.0 kg (11.02 lb)		
Rated line voltage	100 to 240 VAC		
Rated input current	1.5 A (at 100 VAC)		
Rated input frequency	50 to 60 Hz		
Output voltage / current	12 VDC / 5.0 A		

## LUNA[™] Slides

Physical Characteristics

Compatible Slides	LUNA™ 1-Channel Slides	LUNA™ Cell Counting Slides / PhotonSlide™	LUNA™ 3-Channel Slides	LUNA™ 8-Channel Slides	LUNA™ Reusable Slides
Image	ľ				
Material	Luna Counting Slide™: Polystyrene (PS) Other Slides: Poly(methyl methacrylate) (PMMA)			Glass / Aluminum	
Dimensions	25 x 75 x 2.1 mm	25 x 75 x 2.3 mm	25 x 75 x 2.1 mm	25 x 75 x 2.1 mm	25 x 75 x 2.5 mm

# 12. Ordering Information

## Instruments

Cat #	Product	Quantity
L70001	LUNA-FX7™ Automated Cell Counter, Basic Package	1
L70002	LUNA-FX7™ Automated Cell Counter, Advanced Package	1

# **Slides and Reagents**

Cat #	Product	Quantity
L72011	LUNA™ 1-Channel Slides, 50 Slides	1 box
L72012	LUNA™ 1-Channel Slides, 500 Slides	10 boxes
L72013	LUNA™ 1-Channel Slides, Sterile-gamma-irradiated, 500 Slides	10 boxes
L72001	LUNA™ 8-Channel Slides, 50 Slides	1 box
L72002	LUNA™ 8-Channel Slides, 500 Slides	10 boxes
L72003	LUNA™ 8-Channel Slides, Sterile-gamma-irradiated, 500 Slides	10 boxes
L72021	LUNA™ 3-Channel Slides, 50 Slides	1 box
L72022	LUNA™ 3-Channel Slides, 500 Slides	10 boxes
L72023	LUNA™ 3-Channel Slides, Sterile-gamma-irradiated, 500 Slides	10 boxes
L12001	LUNA™ Cell Counting Slides, 50 Slides	1 box
L12002	LUNA™ Cell Counting Slides, 500 Slides	10 boxes
L12003	LUNA™ Cell Counting Slides, 1000 Slides	20 boxes
L12005	PhotonSlide™, 50 Slides	1 box
L12006	PhotonSlide™, 500 Slides	10 boxes
L12007	PhotonSlide™, 1000 Slides	20 boxes
L12011	LUNA [™] Reusable Slide	1 unit
L12014	LUNA [™] Reusable Slide Coverslips	10 units
L72030	Cell Counter Validation Slide-FL	1 unit
L72040	Cell Counter Validation Slide-BF	1 unit
L72041	Cell Counter Validation Slide-BF II	1 unit
T13001	Trypan Blue Stain, 0.4% (200 tests)	2 x 1 mL
T13011	Trypan Blue Stain, 0.4%, Sterile-filtered	2 x 1 mL
L13002	Erythrosin B Stain (200 tests)	2 x 1 mL
L13004	Methylene Blue Stain, 0.02%	2 x 1 mL
F23001	Acridine Orange/Propidium lodide Stain	2 x 0.5 mL
F23011	Acridine Orange/Propidium Iodide Stain, Sterile-filtered	2 x 0.5 mL
F73101	LUNA-FX™ Calibration Beads Kit	2 x 0.5 mL

# CountWire™

Cat #	Product	Quantity
L71001	CountWire™ Basic (1 CountWire™ Data Storage + 1 CountWire™ Verification Key)	1 set
L71002	CountWire™ Verification Key (additional)	1 unit
L71005	CountWire™ Single (1 USB Drive + 1 CountWire™ Verification Key)	1 set

## IQ/OQ

Cat #	Product	Quantity
L74003	LUNA-FX7™ IQ/OQ Protocol	1 сору

## Accessories

Cat #	Product	Quantity
P17001	Thermal Printer	1 unit
P17002	Thermal Printer Refills, Paper and Ribbon	2 x 5 rolls

# **13. Purchaser Notification**

### Limited Use Label License

**Research Use Only** The purchaser of this product should use this product only for research for the sole benefit of the purchaser. By use of this product, the purchaser agrees to be bounded by the terms of this limited use statement whether the purchaser is a for-profit or a not-for-profit entity.

If the purchaser is not willing to accept the conditions of this limited use statement and this product is unused, the Company will accept return of the product with a full refund.

The purchaser cannot resell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party for Commercial Purposes.

Commercial Purposes mean any and all uses of this product and its components by a party for monetary or other consideration, including but not limited to, (a) product manufacture, (b) providing a service, information, or data, (c) therapeutic, diagnostic, or prophylactic purposes, or (d) resale of this product or its components whether or not such product and its components are resold for use in research.

Aligned Genetics, Inc. ("Company") will not claim any consideration against the purchaser of infringement of patents owned or controlled by the Company which cover the product based on the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine, or prophylactic product developed in research by the purchaser in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product.

For any use other than this limited use label license of research use only, please contact the Company or email info@logosbio.com for more information.

### Instrument Warranty

#### Warranty

Aligned Genetics, Inc. ("Company") warrants to the original purchaser ("Purchaser") that the instrument ("Instrument"), if properly used and installed, will be free from defects in materials and workmanship and will conform to the product specifications for a period of one (1) year ("Warranty Period") from the date of purchase. If the Instrument under this limited warranty fails during the Warranty Period, the Company, at its sole responsibility, will: within and up to 30 calendar days of purchase, refund the purchase price of the Instrument to the Purchaser if the Instrument is in original conditions; or, after 30 calendar days of purchase, only replace or repair the Instrument for up to the Warranty Period without issuing a credit.

In no event shall the Company accept any returned instrument (including its components) that might have been used or contaminated in some labs, including but not limited to, HIV or other infectious disease or blood-handling labs. This limited warranty does not cover refund, replacement, and repair incurred by accident, abuse, misuse, neglect, unauthorized repair, or modification of the Instrument. This limited warranty will be invalid if the Instrument is disassembled or repaired by the Purchaser.

In case that the Company decides to repair the Instrument, not to replace, this limited warranty includes replacement parts and labor for the Instrument. This limited warranty does not include shipment of the Instrument to and from service location or travel cost of service engineer, the costs of which shall be borne by the Purchaser. Every effort has been made to ensure that all the information contained in this document is correct at its publication. However, the Company makes no warranty of any kind regarding the contents of any publications or documentation as unintended or unexpected errors including occasional typographies or other kinds are inevitable. In addition, the Company reserves the right to make any changes necessary without notice as part of ongoing product development. If you discover an error in any of our publications, please report it to your local supplier or the Company. The Company shall have no responsibility or liability for any special, incidental, indirect or consequential loss or damage resulting from the use or malfunction of the Instrument.

This limited warranty is sole and exclusive. The Company makes no other representations or warranties

of any kind, either express or implied, including for merchantability or fitness for a particular purpose with regards to this Instrument. To obtain service during the Warranty Period, contact your local supplier or the Company's Technical Support team.

Out of Warranty Service Please contact your local supplier or the Company's technical support team in order to obtain out-ofwarranty service. If necessary, repair service will be charged for replacement parts and labor hours incurred to repair the Instrument. In addition, the Purchaser is responsible for the cost of shipping the Instrument to and from the service facility and, if necessary, the travel cost of a service engineer after 30 calendar days of purchase, only replace or repair the Instrument for up to the Warranty Period without issuing a credit.



Logos Biosystems Aligned Genetics, Inc.

#### **HEADQUARTERS**

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