

LUTHOR

single cell dispenser

LUTHOR single cell dispenser

LUTHOR single cell dispensing Kit

User Guide

Catalog Numbers:

231.01 (LUTHOR single cell dispenser)

231.04 (LUTHOR single cell dispensing Kit)

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CONTACT INFORMATION

Lexogen GmbH

Campus Vienna Biocenter 5

1030 Vienna, Austria

www.lexogen.com

E-mail: support@lexogen.com

Support

E-mail: support@lexogen.com

Tel. +43 (0) 1 3451212-41

Fax. +43 (0) 1 3451212-99

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About This Guide

Before using the LUTHOR single cell dispenser, read Appendix A, p.27 and all other information in this user manual, including any safety and operating instructions. Pay special attention to all warnings displayed on the instrument. Failure to read and follow these guidelines could lead to improper or incorrect usage and damage to the instrument. Improper use could also cause severe personal injury, death, unpredictable results, instrument malfunction, and premature wear to components shortening the lifetime of the instrument. Such actions may void your warranty. Keep the user manual and any other safety and operating instructions provided with the instrument in a safe place, accessible to all users for future reference.

Technical Assistance

For technical questions or assistance in solving a problem:

- Read the User Guide section specific to the step you are performing.
- See Troubleshooting procedures in Appendix D, p.32.

To report any issue with the use of the LUTHOR single cell dispenser, please contact support@lexogen.com. While using the LUTHOR single cell dispenser driver software, click on the tab on the right side of the window titled "FEEDBACK" to report any issue or feedback you might have. If additional assistance is required, contact your authorized Lexogen service provider or the Lexogen technical support at support@lexogen.com.

1. General information

1.1. System Overview

The LUTHOR single cell dispenser is a low-pressure cell dispenser. Lexogen offers a fast, gentle, and traceable solution for single cell dispensing. Intuitive and user-friendly, it does not require extensive maintenance or specific calibration. Compact and flexible, the LUTHOR single cell dispenser easily integrates into an existing workflow and is designed to be operated on a simple bench or under a biosafety cabinet. The LUTHOR single cell dispenser can hold two multiwell plates on its base plate and can support different multiwell plate models and formats.

The technology is impedance-based, thus extremely gentle with cells, preserving their viability and integrity. The LUTHOR single cell dispenser uses a sterile, disposable sensing tip (LUTHOR scd Tip) to sense and record the passage of every cell flowing through the LUTHOR scd Tip aperture (Figure 1).

The LUTHOR single cell dispenser exclusively functions with LUTHOR kits sold by Lexogen GmbH, Lexogen subsidiaries, or Lexogen approved Distributors. The instrument is started by scanning a barcode provided separately, with the acquisition of a LUTHOR kit, via the barcode scanner included with the instrument. If you have not received a barcode, please contact support@lexogen.com.

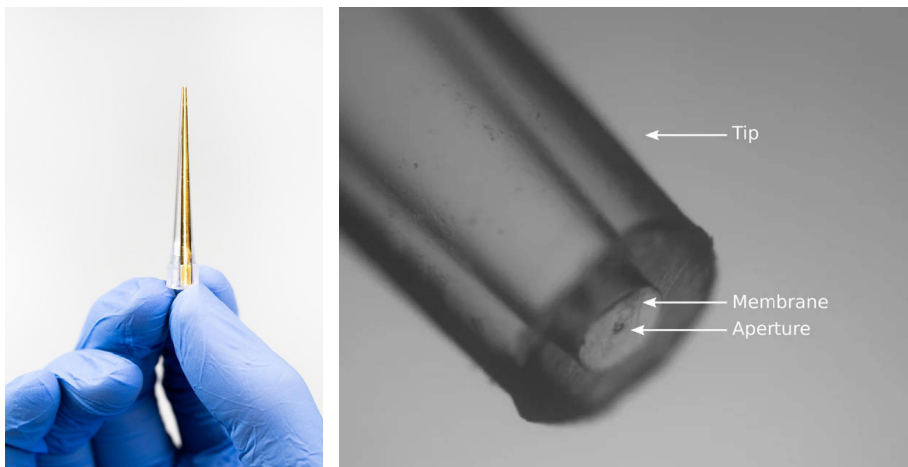


Figure 1. The LUTHOR scd Tip used for impedance based single cell isolation.

1.2. Signal Generation

As a cell passes through the 30 μm LUTHOR scd Tip aperture to flow into the well, it leaves an electrical signature that appears as a unique impedance peak, whereas multiple peaks result from doublets or multiple cells. Cell debris are excluded by filtering out low impedance signals, and cell aggregates are detected by tagging the impedance signals with a high amplitude peak. At the end of the experiment, the impedance measurement of every well of the multiwell plate is processed by the LUTHOR single cell dispenser's analysis software. Wells not meeting the single cell quality control criteria will be rejected by the automated analysis and tagged in red (Figure 2).

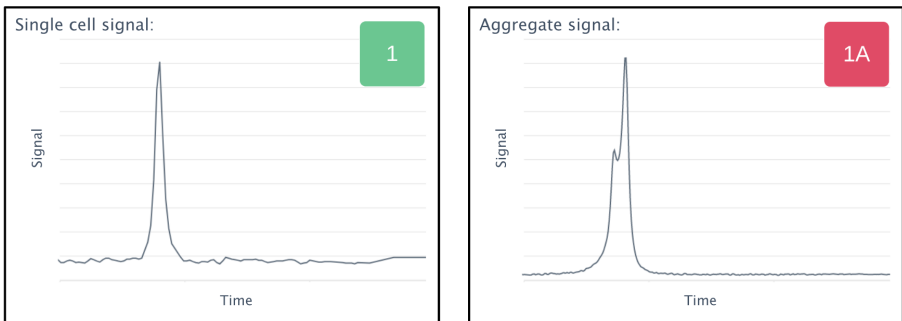


Figure 2. Impedance signature used for single cell detection.

1.3. Instrument and Consumables Description

The whole system solution consists of the:

a) Instrument (Cat. No. 231.01)

Transport box containing:

- 1x LUTHOR single cell dispenser including cable with USB Z port (pipetting head)
- 1x mini PC (Intel NUC 10i5FNKN) without screen, mouse, or keyboard

NOTE: the software is already installed on the provided computer (NUC).

- 1x Base Plate
- 2x PCR-Plate Holder
- 1x Power supply for the LUTHOR single cell dispenser
- 1x Power supply for the mini PC (NUC)
- 1x Supporting Cable CAB1
- 1x Supporting Cable CAB2 (with USB-B) and 1x Supporting Cable CAB2 (with electrical Power Plug Pins, for NUC)
- 1x 4-port USB-C Mini Hub
- Barcode Reader

- Troubleshooting Toolkit
- TSA lock
- 1x Packing Guide (231UI795V0100)

ATTENTION: The following components are required but are not provided by Lexogen:

- Computer screen, including display cable (HDMI type A)
- Computer keyboard
- Computer mouse

Installation, repair, or replacement of the above components is the client's responsibility.

b) LUTHOR single cell dispensing Kit (Cat. No. 231.04)

- LUTHOR scd Tip, the single-use sensing tip
ATTENTION: LUTHOR scd Tip is packaged in a plastic holder within a sterile pouch. Use gloves to manipulate it.
- LUTHOR scd Buffer, the buffer used for cell sample preparation
ATTENTION: Upon arrival, store the LUTHOR scd Buffer in the fridge.
- 1 x single-use cell strainer to remove aggregates during cell sample preparation
- One box of 96 wide bore, filtered mixing pipette tips, sterile
- Pack of 4 washing units, sterile

2. Installation Procedures

Incorrect installation or the operation of a damaged instrument may lead to the exposure of mechanical hazards, electric shock, the spread of fire, explosion, or biohazard. Read through the following instructions carefully before the LUTHOR single cell dispenser installation. Before opening the transportation box, check for any visible external damage to the box. Check also to see if the shock and position indicators suggest incorrect transportation of the instrument. Before operating the LUTHOR single cell dispenser for the first time, carefully read this user guide and contact a Lexogen representative for online training. LUTHOR single cell dispenser is to be used only with the provided power supply model MEAN WELL GSM160A12-R7B and provided mains supply cord in order to guarantee safe operation.

2.1. Site Requirements

Read the instructions in Appendix A, p.27 and ensure that your site is adequately prepared before installing the LUTHOR single cell dispenser. If there is any damage, do not use the instrument but contact a Lexogen representative or our technical support at support@lexogen.com. The instrument is intended for indoor use only and in the following environmental conditions:

- Altitude up to 2,000 m (6,562 ft)
- Temperature 5 °C to 40 °C (41°F and 104 °F)
- Maximum relative humidity 80 % for temperatures up to 31 °C (87.8 °F) decreasing linearly to 50 % relative humidity at 40 °C (104 °F)
- Pollution degree 2
- Electromagnetic environment of domestic class (max 3 V/m, according to IEC61000-4-3)

The main power supply accepts the followings conditions:

- Voltage fluctuations up to ± 10 % of the nominal voltage
- Transient overvoltage up to the levels of overvoltage CATEGORY II
- Temporary overvoltage

Take special care while handling liquids.

2.2. Unpacking

- Take everything out of the box except the LUTHOR single cell dispenser. Verify that all components listed previously are there (See Chapter 1.3. "Instrument and Consumables Description").
- With two hands, gently remove the LUTHOR single cell dispenser out of the box by its base (Figure 3). **ATTENTION:** Do not hold the LUTHOR single cell dispenser by the top part of the instrument.

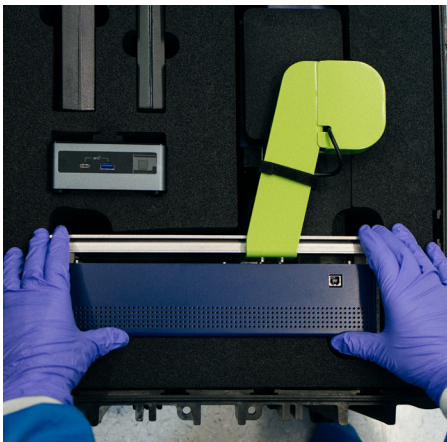


Figure 3. How to lift the LUTHOR single cell dispenser out of the transport box.

ATTENTION: Place the instrument on a stable support. Otherwise, the instrument might fall down, which may cause serious bodily harm and/or severe damage to the instrument.

- The LUTHOR single cell dispenser can be used on a bench or in a biosafety cabinet. Enough space next to the LUTHOR single cell dispenser is required to fit the mini PC (NUC). If placed in a biosafety cabinet, a trolley can be used for the mini PC (NUC). Three electrical outlets are needed for the LUTHOR single cell dispenser, the mini PC (NUC), and the screen.
- Remove the black protective foam around the pipetting head (Figure 4).

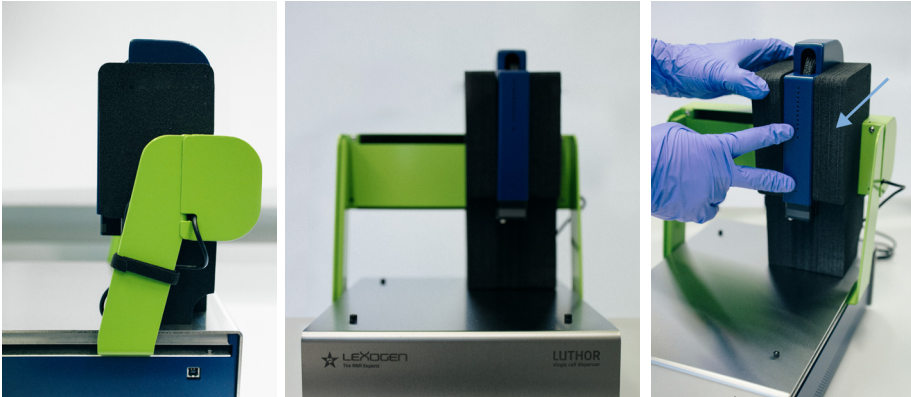


Figure 4. Side view of the protected pipetting head (left), front view of the protected pipetting head (center), and removal of the black protective foam (right).

- Remove the strap holding the USB cable on the right side of the LUTHOR single cell dispenser.
- Assemble the base plate (Figure 5).

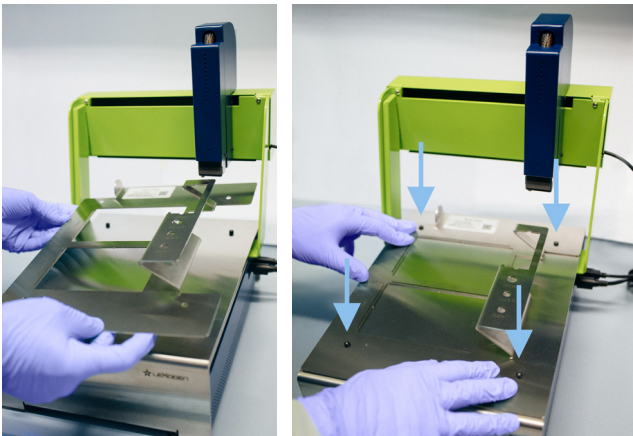




Figure 5. Placement of the base plate on top of the LUTHOR single cell dispenser (left). Clipping of the base plate (right).

2.3. LUTHOR dispenser and mini-PC (NUC) Installation

- Place the mini PC (NUC) close to the LUTHOR single cell dispenser, so that the USB cables can be connected afterward.
- Gather a screen, keyboard, and mouse next to the mini PC (NUC).
- Plug CAB1 into the power supply of the LUTHOR single cell dispenser with the flat side of the plug facing up at the back of the LUTHOR single cell dispenser  (Figure 6, left).
- Plug the USB-B cable (CAB2) with the flat side of the connector facing up into the right side of the LUTHOR single cell dispenser  (Figure 6, right).

LUTHOR single cell dispenser rear panel power supply port



LUTHOR single cell dispenser right side panel USB-B port



Figure 6. Ports of the LUTHOR single cell dispenser.

- Plug the computer screen to the power outlet (not provided).
- Plug the mini PC (NUC) to the power outlet with the power supply. Connect CAB2 (with power socket pins) to the NUC power supply.
- Connect the HDMI cable from the screen to the NUC.
- Connect the USB-B (instrument side) and USB-Z (instrument head) cables from the LUTHOR single cell dispenser to the NUC using the ports located on the rear panel of the NUC (Figure 7).
- Connect the barcode scanner to the NUC (USB-A port).



Figure 7. NUC rear panel ports.


- Connect the provided 4 port USB hub to the USB port located on the front panel of the NUC and connect mouse and keyboard to the USB hub.
- Connect barcode reader.

3. Cell Dispensing with the LUTHOR single cell dispenser

3.1. LUTHOR single cell dispenser driver Startup

- Verify that all cables are correctly connected (refer to: "2.3 LUTHOR dispenser and mini-PC (NUC) Installation").
- Turn on the LUTHOR single cell dispenser by pushing the ON button on the front left side of the instrument. The pumps will make a humming noise.
- Switch on the mini-computer (NUC) and the screen.
- Log on to the account: **Lexogen** with the password: **LUTHORscd**

ATTENTION: A security feature will block the mini-computer (NUC) for 30 minutes after >5 sign-in attempts. If locked, exit the login action, wait 30 minutes, then enter the correct password.

- Open the LUTHOR single cell dispenser driver software by one left click on the icon () located on the upper left side of the screen. The software might take a few minutes to initialize. For each step, the red cross will change to a green tick, and then the "Start" button will appear.

- ✓ Software responding
- ✓ Robot connected (usb CAB2)
- ✓ Acquisition connected (usb CAB3)
- ✓ Software ready

- Click on "Start" to begin the LUTHOR single cell dispenser initialization. At this point, the pipetting head will move. The pipetting head of the LUTHOR single cell dispenser moves to the front left side of the instrument to allow for user alignment.

3.2. Preparing to dispense

3.2.1. LUTHOR scd Tip Installation

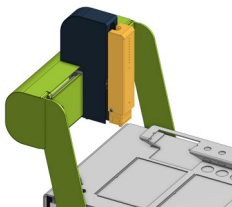


Figure 8.1

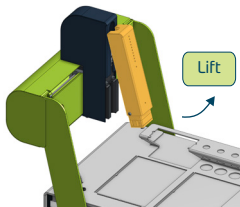


Figure 8.2

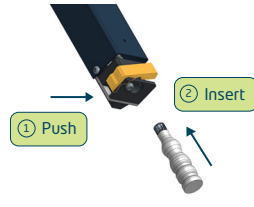


Figure 8.3

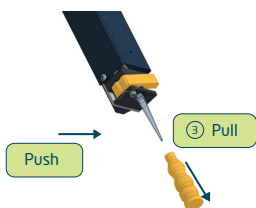


Figure 8.4



Figure 8.5

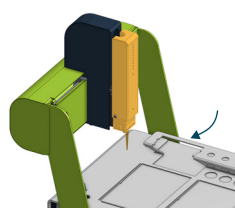


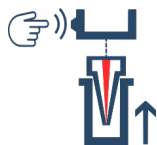
Figure 8.6

Figure 8.1 - 8.6. LUTHOR scd Tip installation on the LUTHOR single cell dispenser's pipetting head.

Open the sterile pouch containing the LUTHOR scd Tip and. Remove the white plastic holder containing the LUTHOR scd Tip.

Open the white plastic holder under sterile conditions by gently lifting the cap.

ATTENTION: Do not touch the LUTHOR scd Tip with your gloves; only manipulate the LUTHOR scd Tip touching the white plastic holder.



Take the pipetting head off its magnetic support with one hand. Use a finger to press the lever on the left lower part of the pipetting head to open the connecting piece before inserting the LUTHOR scd Tip (Figure 8.1 and 8.2).

ATTENTION: Do not apply strength on the sheath linking the pipetting head to the instrument, which could damage the cables and tubing inside it.

Take the LUTHOR scd Tip by its white plastic holder with the other hand and connect the LUTHOR scd Tip with the golden strip positioned towards the front of the instrument. Press firmly (Figure 8.3).

Remove the white plastic holder and release the lever (Figure 8.4 and 8.5).

If the LUTHOR scd Tip is not firmly inserted, it will be ejected.

Place the head back on its magnetic support and click on "Done" (Figure 8.6)

Check the orientation of the LUTHOR scd Tip by confirming that the golden strip of the LUTHOR scd Tip is facing the front side of the LUTHOR single cell dispenser. If that is the case, click on "Done".

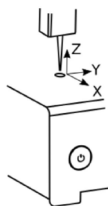


ATTENTION: If the golden strip on the LUTHOR scd Tip is not facing the front of the LUTHOR single cell dispenser, the LUTHOR scd Tip needs to be reoriented. To reorient the LUTHOR scd Tip, insert the LUTHOR scd Tip into the white plastic holder. Then press on the lever and pinch the top part of the LUTHOR scd Tip to maneuver reposition.

3.2.2. LUTHOR scd Tip Alignment



The LUTHOR single cell dispenser pipetting head will move close to the alignment pin.



Align the LUTHOR scd Tip on top of the alignment pin by using the arrows present on the screen. The LUTHOR scd Tip movement is set to 0.5 mm / 0.02 inch increments. This value can be modified to smaller or larger increments.

ATTENTION: Do not exceed 1 mm / 0.04 inch to avoid reaching the limit of the axis.

The height position of the LUTHOR scd Tip is set approximately 2 mm / 0.08 inch higher than the alignment pin. If necessary, this value can be adjusted using the advanced mode.

ATTENTION: Be careful when changing the height position; if the LUTHOR scd Tip hits a hard surface, it may damage the sensor.

Once the alignment is done, click on "Next". The LUTHOR single cell dispenser will move its pipetting head to the back right of the instrument.

3.2.3. Wash Installation

- Fill the washing unit (furnished in packs of 4) with 25 ml of sterile 1x PBS.
- Place the washing unit on the LUTHOR single cell dispenser as indicated. Once done, click on "Next".

3.2.4. Choose Multiwell Plate Type

The LUTHOR single cell dispenser can simultaneously accommodate two multiwell plates.

- Click on "Plate model" to choose the reference of your multiwell plate.
- The default name is YYYY-MM-DD-HH-MM. Click on "Plate label" to change the name of the multiwell plate, avoiding special characters and spaces. An automatic naming can be performed using a barcode reader. When done, click on "Next".

ATTENTION: The label of the multiwell plate needs to match the label used in the LUTHOR single cell dispenser driver software. This is crucial for traceability.

The second multiwell plate is activated using the top-right button.

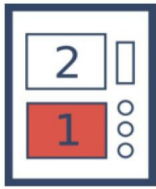



Plate model 
96 WP, 200ul

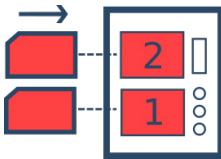
Plate label 
Label_1

3.2.5. Multiwell Plate Placement



Verify that the labeling of the multiwell plate is correct to ensure traceability.

Fill the wells of the multiwell plate with the desired liquid medium (please refer to the corresponding LUTHOR User Guide).



Place the plate in position 1.



Check that the multiwell plate(s) is/are oriented with well A1 situated in the top left.

3.3. Cell Sample Preparation

ATTENTION: Closely follow every step of the protocol. Cell filtration and sample mixing are critical steps for correct cell dispensing.

Take the LUTHOR scd Buffer tubes out of the fridge, quickly spin them down using a tabletop centrifuge before placing them vertically, and wait at least 10 minutes to allow them to reach room temperature.

ATTENTION: Do not warm the LUTHOR scd Buffer at 37 °C / 98.6 °F.



Remove the potential cell aggregates in your sample to prevent clogging of the LUTHOR scd Tip using the strainer (20 μm mesh size) provided in the LUTHOR scd Kit. First, place the cell strainer on a tube (5 ml or 13–15 ml) and prime the cell strainer with 500 μL of culture medium. Next, place the cell strainer on a new tube (5 ml or 13–15 ml) and filter at least 1 ml of the cell suspension.

Check cell concentration, viability, cell size distribution, proportion of cell debris, and proportion of aggregates with your routine protocol and adjust the total cell concentration (live + dead) to 1.5×10^5 cells/ml with culture medium.

ATTENTION: A cell viability below 90% may compromise the results of the experiment.

Add 15 μL of the strained cell suspension at 1.5×10^5 cells/ml in the LUTHOR scd Buffer tube (final concentration: 1.5×10^4 cells/ml).



Use the wide bore 200 μL tip (furnished in box of 96) to mix the cells with the viscous LUTHOR scd Buffer by pipetting up and down 30 times.

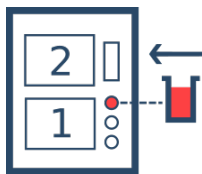
ATTENTION: Avoid the generation of air bubbles. They will interfere with the impedance measurement.



If air bubbles are created during mixing, flick the tube downward to remove the air bubbles. When done, click on "Next".

ATTENTION: Do not spin down the tube in a centrifuge or minifuge.

3.4. Loading



Select the sample tube position (0.5 ml by default in position 3). Then place the cell sample tube in the corresponding position on the tube rack of the LUTHOR single cell dispenser. For low cell number applications, Lexogen provides a custom configuration to use tubes with lower volume.

ATTENTION: Open the cap of the sample tube to allow the sample to be loaded in the LUTHOR scd Tip.

Adjust the loading time according to the type and the number of multiwell plates to process and the target number of cells per well (Table 1).

Multiwell plates	Recommended loading time
1x 96 multiwell plates	60 sec
2x 96 multiwell plates	120 sec
1x 384 multiwell plates	240 sec

Table 1. Recommended loading times for single cell dispensing in each well

ATTENTION: Do not load the sample in the LUTHOR scd Tip for more than 240 sec. If you need to dispense more volume, first dispense the totality of the sample loaded in the LUTHOR scd Tip and then reload.

Enable the histogram generation (activated by default) to perform a 30 seconds analysis of the sample to generate preliminary values of the impedance signal distribution of the sample.

Click on "Done" and then on "Load" to load the cell sample in the LUTHOR scd Tip.

ATTENTION: When viewing "Live values" during loading, the pressure should be between -150 and -100 mbar, and impedance should be between 30,000 and 50,000 ohms. If this is not the case, check the table below (Table 2).

Impedance value [ohm]	Meaning
30,000 - 50,000	Normal range
< 30,000	The LUTHOR scd Tip may be not correctly inserted. Refer to 3.2.1 LUTHOR scd Tip Installation, to reposition the LUTHOR scd Tip in the pipetting head. If the same value appears, change the LUTHOR scd Tip.
> 50,000	The LUTHOR scd Tip might be clogged. Retry loading. If the same value appears, change the LUTHOR scd Tip. Make sure the cell sample is correctly strained.
0	No acquisition of the impedance. At the end of the loading, leave the LUTHOR scd Tip on the instrument and restart the mini-computer (NUC) and the LUTHOR single cell dispenser. Resume at the loading step in the software and proceed with a 5 second loading.
Saturation	Refer to "Troubleshooting" section, Appendix D, p.32.

Table 2. Impedance live value and meaning

- If the histogram generation is enabled, the LUTHOR single cell dispenser will measure approximately 20 events to generate and plot indicative values about the sample:.

Histogram

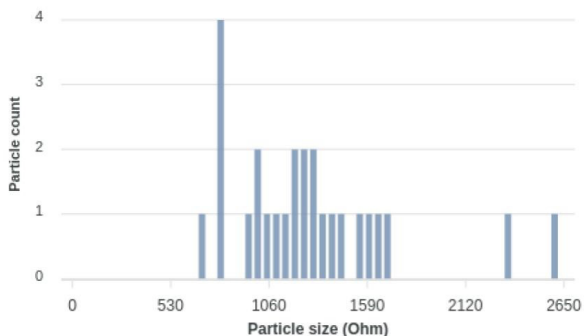


Figure 9. Example of histogram generated at the loading step showing the impedance signal distribution of the cells of the loaded sample.

The following metrics of the cell dispenser are estimated:

- The Tcc (average time between two events) expresses the concentration of the sample.
- The PFR indicates the percentage of wells containing a single cell.
- The total time to dispense one single cell in every well of the multiwell plate.

ATTENTION: If you are outside the 2 to 5 seconds Tcc range, the sample may not be appropriately mixed or at the recommended cell concentration.

With a low Tcc, the time required for the dispense will decrease, and the number of wells with surplus cells will increase. Cell dispensing can be performed, but the single cell efficiency might be lower.

With a high Tcc, the time required for the dispense will increase, and the number of wells with surplus cells will decrease. Cell dispensing can be performed, but a reloading of the sample might be needed to have enough sample to dispense the full multiwell plate.

3.5. Wash

A washing step of 5 cycles is performed in the washing unit filled with 1x PBS to clean the external surface of the LUTHOR scd Tip from the viscous solution.

- Click on "Wash" to initiate the washing of the external surface of the LUTHOR scd Tip.

3.6. Starting and Monitoring the Cell Dispense

- Choose the dispense mode. The "Cell count" mode is used to specify the number of cells to dispense per well.

3.6.1. Cell Detection Parameters

The "Cell count" mode requires a thresholding to filter out the cell debris signals from the detection of the cell to have a correct triggering during the dispense. Note that the threshold does not influence the recorded impedance signal but affects the count of cell events and, therefore, the accuracy of the dispense. The threshold can be modified in the post-treatment analysis, if necessary.

- The threshold is specific to a cell line and needs to be determined experimentally (cf. Figure 10 and 11). For most mammalian cells bigger than 10 μm , the recommended threshold is 200 ohms.

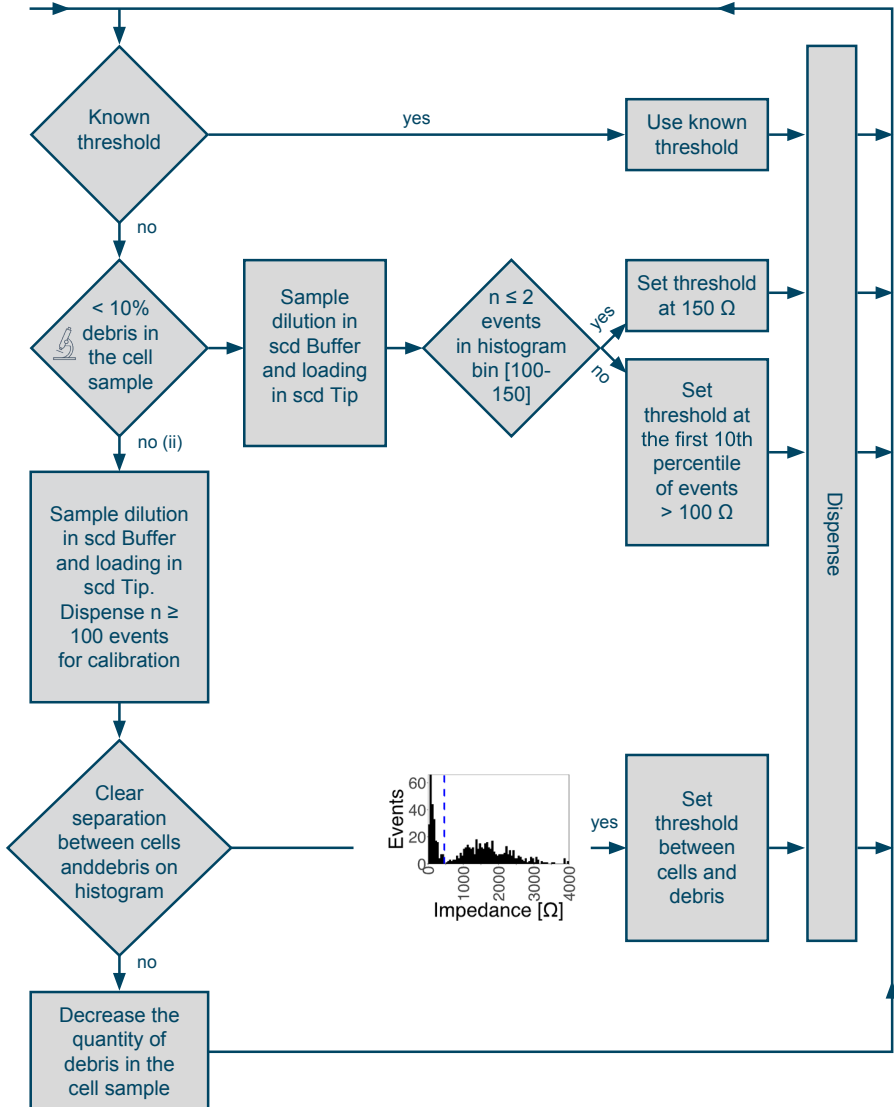


Figure 10. Guidelines to define the threshold between noise/debris and cells.

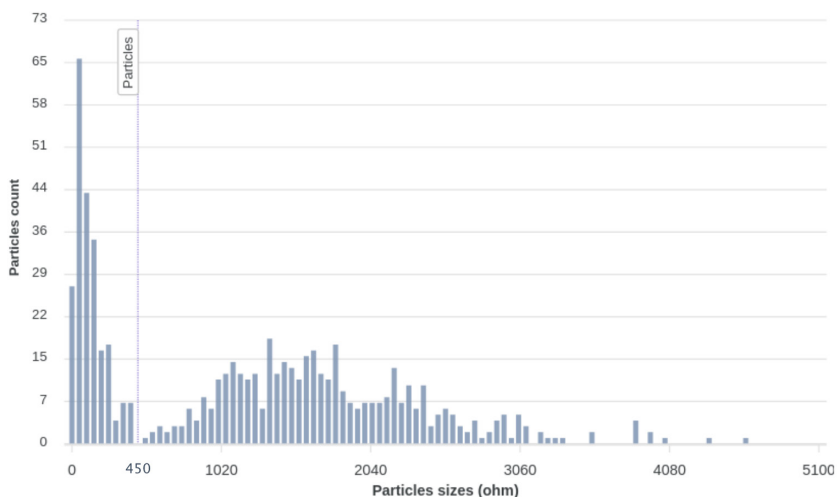


Figure 11. Example of the distribution of the impedance signal (in ohm) of >400 cell events obtained with a HEK293 cell sample after analysis in the LUTHOR single cell dispenser analysis software. The "particle threshold" used to distinguish cell debris from cells was set at 450 ohms.

ATTENTION: Document the cell detection threshold used.

- In the "Cell count" box, leave 1 for single cell dispensing or specify the desired number of cells to dispense per well.

3.6.2. Cell Dispensing

- If two multiwell plates are loaded, select the desired plate position.
- Select the wells to dispense into with one left click on the first well and one left click on the last well of your selection. The software will automatically select the wells in between.
- Click on "Dispense" to start the dispensing process.

ATTENTION: Control "Live values", the pressure should be between 10 and 20 mbar.

The impedance signal can be checked by one right click on the well already dispensed.

ATTENTION: If LUTHOR scd Tip is left at rest for several minutes in between dispensing, go back to the "Wash" step and perform another wash cycle before resuming to the dispense.

3.7. End of Dispense

After dispensing, several options are available:

- "Change tip" to use another sample.
- "Change multiwell plate" to dispense in a new multiwell plate.

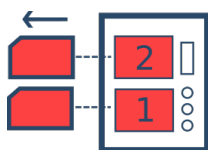
- "Reload sample" to reload the sample in the LUTHOR scd Tip, if the loaded volume in the LUTHOR scd Tip is insufficient to continue dispensing.
- "Change detection settings" to modify the dispensing parameters (cf. 3.6.1. Cell Detection Parameters).

To end the experiment, click on the "Finish" button.

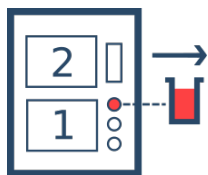
ATTENTION: When the experiment is classified as finished, the folder named with the multiwell plate cannot be modified with new dispensing data.

3.8. Shutdown

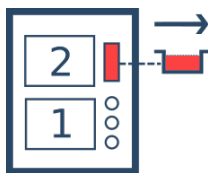
When dispensing is complete, terminate with the following steps:



Store the multiwell plate(s). Seal and gently remove the multiwell plate(s) from the LUTHOR single cell dispenser.



Remove the sample. Close the tube used for loading and remove it from the LUTHOR single cell dispenser.



Remove the wash unit. Remove the washing unit carefully to avoid any liquid spill on the LUTHOR single cell dispenser.



Remove the LUTHOR scd Tip. Take the pipetting head off its magnetic support with one hand. Use a finger to press the lever on the left lower part of the pipetting head to open the connecting piece, pinch, and pull the top part of the LUTHOR scd Tip to remove the LUTHOR scd Tip.


ATTENTION: Do not pull the LUTHOR scd Tip without pressing on the lever; this might damage the connector of the pipetting head.


Quit the LUTHOR single cell dispenser driver software and turn off the LUTHOR single cell dispenser.



4. Analysis

4.1. Results Visualization

To visualize the results of your experiments, open the LUTHOR single cell dispenser analysis software on the mini-computer (NUC) or your personal computer.

- Open the LUTHOR single cell dispenser analysis software by clicking the icon on the upper left side of the screen (). The software might take a few minutes to load.
- Select the data source folder.

ATTENTION: If the LUTHOR single cell dispenser analysis software was open while dispensing, refresh the multiwell plates list by clicking on the refresh button in the top right of the window ().

- Perform the automatic cell detection in the dispensed multiwell plate by clicking on: 
- Visualize the results of the experiment by clicking on: 

Three panels will appear: the histogram of the particle-size distribution, the detection parameters, and the representation of the results on the multiwell plate.

- Click on the "Edit" button in the detection parameters panel.
- Modify the value of the "Particle threshold" with the value used for the dispensing of this multiwell plate and click on "Save".
- On the panel representing the results on the map of the multiwell plate, right click on wells to visualize the impedance signals and control the automatic cell detection (cf. next section 4.2.2 Impedance Signal Interpretation).

4.2. Automatic Analysis

The LUTHOR single cell dispenser analysis software performs an automatic analysis of the impedance signal in every well. The automated analysis is updated when the detection parameters are changed to refine your results.

ATTENTION: We recommend that the user manually verifies the automatic analysis performed by the LUTHOR single cell dispenser analysis software.

4.2.1. Thresholding

The LUTHOR single cell dispenser analysis software uses two thresholds ("Particle threshold" and "Aggregate threshold") to post-process the acquired impedance signals during the dispense of the multiwell plate to exclude the wells not meeting the quality criteria.

The software uses, by default, the "Particle threshold" set by the user for the cell dispense (to distinguish the cell debris from the cells' impedance signal). The automatic cell detection will be updated by changing the "Particle threshold".

If the "Particle threshold" is set too low, the software may interpret lower impedance signals as cells. This can lead to an increase in excluded wells due to misclassified events like noise or cell debris, ultimately resulting in a high cell count.

If the "Particle threshold" is set too high, the software will ignore the lowest impedance signals of some cells during dispensing. This results in an increase in excluded wells, which may be marked as empty, potentially overlooking small cells.

The software, by default, is not applying an "Aggregate threshold" (to distinguish the cells from the aggregates' impedance signal). The automatic cell detection will be updated by changing the "Aggregate threshold".

The lower the "Aggregate threshold", the more wells with the highest impedance signals will be excluded as they are considered to contain an aggregate (some of the excluded events being large cells). The "Aggregate threshold" is defined using the microscopic observation of the cell sample before dispensing to quantify the proportion of aggregates (cell doublet, cell clumps). For example, if the cell sample contains 5% of aggregates, 5% of the highest impedance signals should be excluded by setting the "Aggregate threshold" accordingly.

4.2.2 Impedance Signal Interpretation

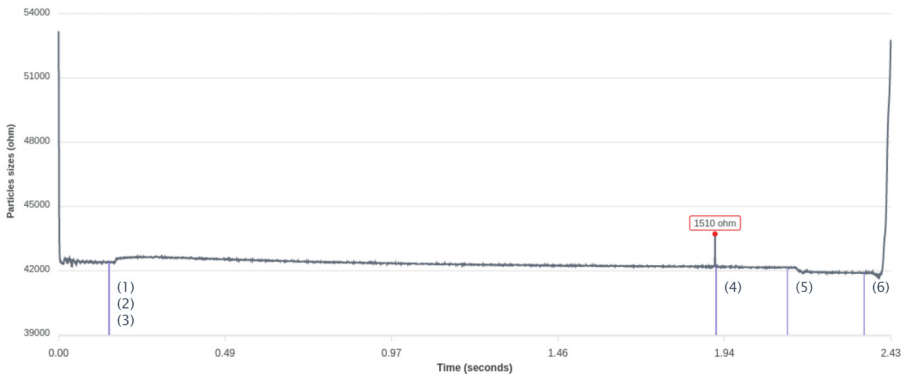


Figure 12. Typical impedance signal obtained with a single cell dispense into a well over time.

The impedance signal measure is composed of different phases:

- (1) The moving of the LUTHOR scd Tip on top of the well
- (2) The LUTHOR scd Tip entering the well
- (3) The activation of the pump to reach the dispensing pressure
- (4) The peak detection window ended by the cell detection or by reaching the timeout
- (5) The inactivation of the pump to reach atmospheric pressure
- (6) The exiting of the well

Those different phases can be visualized on the impedance signal by selecting the option in Settings -> Other -> Show events in well charts.


4.3. Reporting

The LUTHOR single cell dispenser analysis software report provides immediate and traceable proof of clonality. Analyze the impedance signal in each well to control the automatic analysis.

A monoclonality report can be created for each experiment. Click on the button "Actions" in the upper right corner to export the data in .pdf files or .csv files.

- The .pdf report contains the particle size distribution, the map of the multiwell plate with the result per well, and the particle detection setting.
- The plate_results.csv file contains the map of the multiwell plate with the result per well.
- The detected_events.csv contains the impedance values of all the events detected per well.
- The dispense_events.csv contains the time of the event triggering the end of the well's dispense.
- The single_cells.csv contains the coordinates of the well dispensed with a single cell.

These results files can be saved, for example, in the Documents or Downloads folder but should not be saved in the folder containing the raw impedance data.











Visualize the impedance signal of the individual wells by clicking on the multiwell plate map on each well. Click-and-drag zoom on the impedance signal using the left mouse button. The displayed measure of the impedance can be exported in different formats (.svg, .png, and .csv) by clicking on the "Save" icon ()

If necessary, click "Actions" in the upper right corner to manually correct the automatic analysis.

5. Appendix A: Important Safety Information

Symbols on the Instrument

Each safety label on the instrument contains an alert symbol that indicates a type of potential safety hazard.

Symbol	Indication
	Consult the product documentation. Read and understand the operator's manual before using this equipment.
	Potential electrical-shock hazard from a high-voltage source. All safety instructions must be read and understood before proceeding with the installation, maintenance, and servicing of all modules.
	Risk of electrical shock, do not open.
	Indoor use only
	WEEE compliant
	Location of a fuse.
	DC current
	The instrument or disposable manufacture date.
	Compliance with Chinese RoHS Pollution Control Requirements.
	CE marking

5.1. Warnings and Precautions

The instrument is designed for safe use when installed correctly and operated by trained personnel following general safety practices and the instructions in this user manual. Any use of the instrument other than that described in this manual may result in danger to the user. The guidelines in this section explain the potential risk associated with using this instrument and provide important supplemental safety information to minimize the risks. Follow the instructions carefully to protect yourself, others, and the equipment from potential hazards and create a safe work environment. Use this instrument only as specified to avoid damage to equipment and injury to personnel. Always follow local working area safety instructions and laboratory policies, standards for health, safety, and prevention of accidents. Contact the local authorities governing electrical power supply, building constructions, maintenance, or safety for more information about the safe installation and operation of the instrument.

Do not use the instrument if:

- It has been opened or disassembled
- It has been dropped or damaged
- It has damaged or broken parts
- It has a damaged power cable
- If an object has entered into the instrument

5.2. Hazard Levels

Signal words are used to identify safety and property damage messages. The following signal words are used throughout this user manual.

- **WARNING!** indicates a potentially hazardous situation, which, if not avoided, could result in death or severe injury.
- **CAUTION!** indicates a potentially hazardous situation, which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **NOTICE** addresses practices or information unrelated to personal injury but may lead to property damage.

5.3. Electrical Shock and Fire Hazard

WARNING! Electrical Shock Hazard. Do not remove the covers. Removing the covers might cause injury or malfunctioning of the system. The covers need not be removed for routine maintenance or operation of the system. Only authorized personnel are allowed to remove any cover of the instrument. Never push a foreign object through an opening into the instrument. Ensure that the correct protective earth (grounding) is connected to the main power outlet. The instrument is equipped with a three-wire electrical plug, do not try to insert the plug into a non-grounded power outlet. Do not use any other cables than provided with LUTHOR single cell dispenser.

In case of failure and for emergency stop, the main power supply plug shall be unplugged. Keep the power plug accessible at all times.

This instrument is protected by a fuse with the following characteristics: 250 V, 10 A, Type T, format 5x20.

WARNING! Fire Hazard. Do not use the instrument in areas classified as hazardous locations, for example, in oxygen-laden environments. If flames or smoke appear, immediately switch off the power supply, unplug the instrument from the electrical outlet, and contact Lexogen Technical Support.

WARNING! Electrical Shock and Fire Hazard. Do not allow liquids to enter the interior of the instrument. Protect the instrument against accidental spillages and splashes. Clean up spillages immediately. Do not operate the instrument if liquids have entered the instrument. Do not use the instrument in a wet and damp location. Avoid areas with high humidity or condensation. Before cleaning, unplug the instrument.

5.4. Biological Safety

This instrument was primarily designed to be used with nonpathogenic samples in biosafety level 1 environments (BSL-1) or lower.

Nevertheless, keep in mind to handle your sample with the greatest care following the required safety regulations. Your company biosafety officer must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are suitably trained and aware of applicable Safety Data Sheets (SDS) or other regulatory documents related to your sample used with LUTHOR single cell dispenser. In view of those documents, the operator must wear personal safety equipment accordingly. Disposal of the sample must also be in accordance with all national, state, and local health and safety regulations and laws. If your sample has spilled on the instrument, care must be taken to clean the instrument thoroughly. It should be avoided to continue to handle the instrument before cleaning.

5.5. Chemicals Safety

Isopropanol and ethanol (between 70% to 100%) are recommended to clean and disinfect LUTHOR single cell dispenser. Please also observe the applicable Safety Data Sheets (SDS) when manipulating those substances and wear personal safety equipment accordingly. Disposal of wastes must also be in accordance with all national, state, and local health and safety regulations and laws.

WARNING! Hazardous chemicals. If you use hazardous chemicals or chemicals which become hazardous after completing the protocol run, follow the required safety regulations. Always wear safety glasses, gloves, and a lab coat. The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instru-

ment operators are not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Materials Safety Data Sheets (MSDS) or other regulatory documents. Disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.

5.6. Servicing and Transportation

Improper or incorrect servicing or repair of the instrument can cause hazards to users, lead to unpredictable results, cause instrument malfunctions or damage, as well as premature wear and tear and reduced life of the instrument. Do not service the instrument yourself. Servicing and repair must be performed by qualified service personnel.

Using unauthorized parts can cause malfunctions of the instrument and impair results. Lexogen does not honor any warranty or accept any responsibility for instrument failure or damages resulting from the use of inappropriate parts.

5.7. Repackaging and Shipping After Usage

The instrument needs to be decontaminated before packing it into its dedicated carrying suitcase. Please fill in and sign the decontamination sheet provided on this page: www.lexogen.com/docs/luthor-dispenser

Once decontamination has been performed, and decontamination sheet has been filled in, please reach support@lexogen.com for instructions on how to repackage the instrument appropriately and then proceed to shipment.

The instrument should be transported with care in packaging specified by Lexogen. Internal damage can occur if the instrument is subjected to excessive vibration or is dropped. If you have questions regarding proper decontamination or shipment, contact Lexogen (support@lexogen.com) for assistance.

5.8. Mechanical hazard



During the operation of the instrument, there exists a pinching hazard between the extremity of the LUTHOR scd Tip and the instrument work surface. Therefore, be careful not to place your hands on the instrument during the dispensing process or at any time the instrument is moving.

6. Appendix B: Maintenance

Read the Appendix A, p.27. Do not service the equipment yourself. Service shall be carried out only by trained experts authorized by Lexogen. An improperly serviced instrument may lead to severe personal injury or death by exposure to mechanical hazards and the risk of electric shock. Always wear protective gloves and eyewear to protect against potential biohazard exposure. Before cleaning, unplug the instrument. Do not use liquid or aerosol cleaning agents, always use a damp cloth.

7. Appendix C: Disinfection and Placement Under Biosafety Cabinet (BSL-1 Only)

- Remove the base plate of the LUTHOR single cell dispenser.
- Wipe down the external surfaces of the instrument, the base plate, and cables using a lint-free cloth damp with an appropriate solution (e.g., 70% ethanol or 70% isopropanol).
- Place the LUTHOR single cell dispenser on a trolley in front of the biosafety cabinet.
- Repeat the previous wiping on all the external surfaces of the LUTHOR single cell dispenser, base plate, and cables, and place the LUTHOR single cell dispenser under the biosafety cabinet.

It is recommended to use the correct disinfectant for the specific pathogen and to decontaminate surfaces in contact with biohazardous samples.

The LUTHOR single cell dispenser is ideally placed on the right side of the biosafety cabinet; placement on the left might require a USB type A extension. Do not cover the front grid of the biosafety cabinet with the LUTHOR single cell dispenser and leave a minimum distance of 15 cm / 5.9 inches from the front window.

Leave a minimum distance of 10 cm / 3.9 inches from the back and lateral walls of the biosafety cabinet.

Ideally, the cables connected to the LUTHOR single cell dispenser should go through a dedicated opening on the biosafety cabinet wall. Alternatively, they can exit the biosafety cabinet through the front window.

8. Appendix D: Troubleshooting

ID	Action (perform the action)	Expected result (go to next line if result as expected)	Observation (possible issues to be observed)	Cause	Failure Mode	Fix
1	Manually move all the axes at the mid-way position	Axes should be free and can be placed mid-way on all the axes	Impossible to move the axis	Mechanical blockage	LUTHOR single cell dispenser collides with an external object	IQ procedure
					Casing blockage, slider blockage, motor blockage	Contact support
2	Plug the LUTHOR single cell dispenser into its power supply and connect its two USB cables to the NUC	LUTHOR single cell dispenser power supply can be inserted with reasonable force	Impossible to insert the power supply plug	Wrong orientation	Wrong orientation	Ensure the connector's correct orientation
3	Turn LUTHOR single cell dispenser ON by pressing its power button	The power button turns blue	Front Power button does not light ON	The LUTHOR single cell dispenser main cable is not connected	LUTHOR single cell dispenser is not power supplied	Plug the main cable (CAB1) into the power outlet
			Front Power button does not light up, and the power supply makes an unusual noise	12V power supply connector is connected upside down	LUTHOR single cell dispenser is not power supplied	Ensure the connector's correct orientation
			Front power button does not light up, and the power supply green LED does not light up	12 V power supply default	LUTHOR single cell dispenser is not power supplied	Change the power supply and the main's cable with a replacement part
			Front power button does not light up	The wall outlet is not powered	LUTHOR single cell dispenser is not power supplied	Connect the main cable (CAB1) into another outlet, and test the outlet with another device (e.g., a lamp)

ID	Action (perform the action)	Expected result (go to next line if result as expected)	Observation (possible issues to be observed)	Cause	Failure Mode	Fix
3	Turn LUTHOR single cell dispenser ON by pressing its power button	The power button turns blue	Front power button does not light up	The LED of the button is not functioning	LUTHOR single cell dispenser is power supplied but there is no blue light on the power button	Contact support
			Front power button does not light up	Blown fuse	LUTHOR single cell dispenser is not power supplied	Contact support
			Front power button does not light up	Wrong cables	LUTHOR single cell dispenser is not correctly powered	Contact support
4	Observe the ZHead LED (through the headcover)	Red and blue LEDs light up	No red LED	ZHead not power supplied	-	Contact support
			No blue LED	ZHead firmware not flashed	-	Contact support
5	Listen to the noise of the pumps	A constant electrical-motor-like noise of ~50 dB can be heard in a quiet environment	Pumps do not make any sound	Firmware power up failure	Pumps do not start because the firmware is not flashed or did not power up	Turn LUTHOR single cell dispenser off and turn it on, twice if necessary
				Disconnected pumps connectors	Pumps do not start because the pumps connectors are not connected or defective	Contact support
6	Try again to displace the axis manually	All axes should be blocked	One or more axes are loose	Undriven axis	Motor misconnection	Contact support
					Undriven belt / loose pulley	Contact support
					Mechanical defect of a motor	Contact support

ID	Action (perform the action)	Expected result (go to next line if result as expected)	Observation (possible issues to be observed)	Cause	Failure Mode	Fix
7	Open the LUTHOR single cell dispenser driver software	Launch the LUTHOR single cell dispenser driver and observe the first screen. The labels down to "Software ready" are ticked with a green mark (within 30 sec)	Observe the "USB CAB2 & CAB3" tick in the first software panel	USB cables (CAB2 or CAB3) unplugged	LUTHOR single cell dispenser is not connected to the software	Make sure both USBs are plugged
			Observe the "USB CAB2 & CAB3" tick in the first software panel	USB/COM port corrupted	LUTHOR single cell dispenser is not connected to the software	Unplug both USBs, quit the LUTHOR single cell dispenser driver software, restart the computer, and replug the two USBs
			Observe the "USB CAB2 & CAB3" tick in the first software panel	USB cables (B or Z) damaged	LUTHOR single cell dispenser is not connected to the software	Change the USB-B cable (CAB2)
8	Run a hardware Self-test with a dummy tip inserted in the LUTHOR single cell dispenser pipetting head	Self-test results without errors or warnings	No text is written in the console	Connection/communication problem	Broken USB Port on motherboard	Contact support
			No movement on the Z axis (to reach zero)	Endstop Z misconnection	-	Contact support
				Endstop Z defective	-	Contact support
				Motor misconnection	-	Contact support
				Motor defective	-	Contact support
			Consecutively to the Z axis movement, the XY axis does not move simultaneously (to reach zero)	Endstop X or Y misconnection	-	Contact support
Endstop X or Y defective	-	Contact support				

ID	Action (perform the action)	Expected result (go to next line if result as expected)	Observation (possible issues to be observed)	Cause	Failure Mode	Fix
8	Run a hardware self-test with a dummy tip inserted in the LUTHOR single cell dispenser pipetting head	Self-test results without errors or warnings	Strange noise at the end of the axis displacement; the axis goes to a stop, and the motor lock is heard	Endstop / magnet misplacement or axis encumbrance	-	Contact support
			Error on the axis displacement values	Missed step, mechanical encumbrance, mechanical defect, intermittent connection	Warning on XYZ movements	Contact support
			Error on pressure values, both positive and negative	Dummy tip not correctly connected	Leak on the dummy tip	Disconnect and reconnect the dummy tip firmly and air-tight
			Error on pressure values, both positive and negative pressure value	Leaky manifold	The manifold was incorrectly mounted, the glue was missing on the vias, unmounted O-ring	Contact support
			Error on a pressure value, only one pressure value	Defective pump	Jammed valve, broken / blocked motor, dusty membrane	Contact support
				Pressure divider badly tuned	Throttle valve untuned	Tune the divider
				The pneumatic circuit is open (tube, fitting, throttle valve)	Tubing disconnected, torn fitting, torn throttle valve	Contact support
			Error on the rise time (rise time > 50 ms)	Pinch in the pneumatic tube	-	Contact support
				MoleEye defective	-	Contact support

ID	Action (perform the action)	Expected result (go to next line if result as expected)	Observation (possible issues to be observed)	Cause	Failure Mode	Fix
9	Initiate a 60 sec loading in the driver software, observe LUTHOR scd Tip positioning	The LUTHOR scd Tip inserted correctly into the loading tube	LUTHOR scd Tip is not correctly inserted into the tube	Tube wrongly placed, base plate is incorrectly mounted, base plate tolerances out of specifications	Base plate tolerances are out of specification	Change the base plate
				COM connection/ Software issue	Software not responding	Restart the LUTHOR single cell dispenser driver software
10	Initiate a loading of 60 sec (without histogram) in the driver software. At the end of the loading process, observe the liquid loading level and the impedance readout	After loading, the LUTHOR scd Tip (30 µm aperture) liquid height is 14 mm +/- 5 mm	The liquid level is below 9 mm	Bad pneumatic connection between the tip and the connector	The connection between the tip and the instrument connector is not sufficiently tight and creates a pressure drop that prevents efficient loading	Try to remount the same LUTHOR scd Tip firmly. Press with a sufficient force (similar to regular manual pipette - tip insertion).
				LUTHOR scd Tip clogging	Clogging of the aperture prevents loading	Try to reload and if not successful, change the LUTHOR scd Tip
				The tube conveying pressure inside the instrument is pinched	No more pressure is applied to the LUTHOR scd Tip	The instrument requires a corrective maintenance according to ID8
				LUTHOR scd Tip defects	Burrs on the tip outlet break the tightness and prevent loading	Change LUTHOR scd Tip

ID	Action (perform the action)	Expected result (go to next line if result as expected)	Observation (possible issues to be observed)	Cause	Failure Mode	Fix
10	Initiate loading for 60 seconds (without histogram) in the Driver software. At the end of the loading process, observe the liquid loading level and the impedance readout	After loading, the LUTHOR scd Tip (30 μm aperture) liquid height is 14 mm +/- 5 mm	The liquid level is below 9 mm	LUTHOR scd Tip defects	The inner electrode is damaged with the spring terminal turn, breaking tightness, and preventing loading	Change LUTHOR scd Tip
					The aperture is not correctly ex-cized preventing loading	Change LUTHOR scd Tip
					Electrical contact loss because of a incorrect tip installation (gold electrode not in the right position)	Reposition LUTHOR scd Tip
		After loading, the impedance readout is comprised in the following range: $30 \text{ k}\Omega > Z < 65 \text{ k}\Omega$	Impedance readout is 65535 Ω (signal saturation)	Signal saturation due to an electrical contact issue	Electrical contact loss because of damage to the LUTHOR scd Tip electrode	Change LUTHOR scd Tip
					Electrical contact loss due to a defect in the ZHead connector	Contact support
					Impedance readout deactivated due to electrostatic discharge on the impedance acquisition system.	Leave the LUTHOR scd Tip on the LUTHOR single cell dispenser, restart the computer and the LUTHOR single cell dispenser. Re-open the LUTHOR single cell dispenser driver and resume at the loading step
Impedance readout is exactly 0 Ω	No acquisition of the impedance signal					

9. Appendix E: Technical Specifications

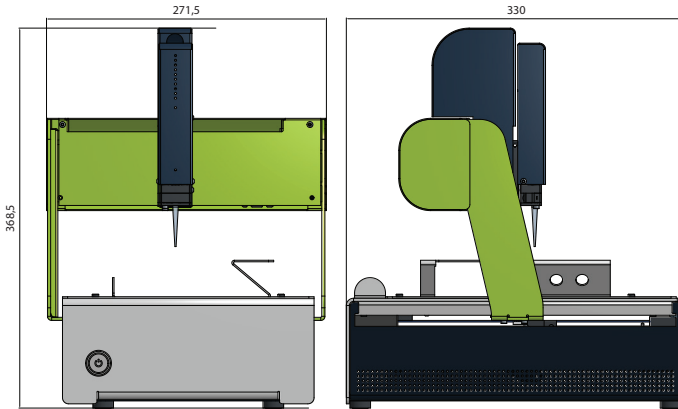
Model: LUTHOR single cell dispenser

Physical Characteristics	
Instrument Size	328 mm x 271.5 mm x 369 mm
Instrument weight	11.1 kg
Electrical Characteristics	
Power supply	External, Meanwell GSM160A12-R7B, 138 W, 12 V, DC 11.5 A certified IEC60601
Input voltage (External power supply)	100 - 240 V~, 50/60 Hz Accepts 10 % voltage fluctuation, Transient overvoltages cat. 2, Temporary overvoltages
Input current (External power supply)	max 2.0 A
Input voltage (Instrument)	12.0 V
Max. power (Instrument)	40 W
Main Fuse	250 V, 10A, Type T, Format 5x20
Process	In use with LUTHOR scd Tip 30 µm aperture
Consumables	
Well plates compatibility	1. 96 WP, ANSI/SLAS 1-2004 and 4-2004 formats 2. 384 WP, ANSI/SLAS 1-2004 and 4-2004 formats
Loading duration in function of well plate	96 WP: 60 seconds 384 WP: 240 seconds
Loading volume	Per 30 second loading time at 1.5×10^4 cells/ml: 10 µL, 200 cells
Loading tube	Sarstedt 0.5ml, 150µL, (pos. 3) Sarstedt 2.0ml, 500µL, (pos. 3)
Cleaning & decontamination	Method: wipe Agent: Isopropanol 70 % (water 30 %) Isopropanol ≥98 % Ethanol 70 % (water 30 %) Ethanol ≥98 %

Performance	
Particle range detection capability	Dielectric, from 8 µm to 25 µm
Time to dispense a 96 well plate	≤10 minutes (provided Tcc <4sec) ≤13 minutes (provided Tcc <6sec)
Time to dispense a 384 well plate	≤40 minutes (provided Tcc <4sec) ≤52 minutes (provided Tcc <6sec)
PFR (Plate filling rate) or efficiency	≥70 % (provided Tcc >2.5 sec)
Monoclonal reliability	≥90 %
Missed/corrupted data points	<1 % per plate (only accounted for point series n >6)
Communication protocol reliability	N/A
Fleet averaged MTBF	N/A
Standards & Certifications	
Electromagnetic compatibility	FCC part 15 B
Safety	IEC61010
Materials	RoHS
Waste	WEEE
Environmental Conditions	
Altitude	Up to 2,000 m
Temperature	+5 °C to +40 °C (41 °F to 104 °F)
Maximum relative humidity	80 % for temperature up to 31 °C decreasing linearly to 50 % relative humidity at 40 °C
Pollution	Degree 2
Electromagnetic environment	According to IEC61000-4-3, domestic class, max 3 V/m

9.1. Size & Weight

The LUTHOR single cell dispenser exhibits a footprint that can conveniently fit in a biosafety cabinet or on any lab bench. Its weight and dimensions also allow transport in airway checked baggage.



All unit in mm.
Total weight: 11.1 kg

9.2. Base Plate Capacity

Standard elements:

- 2 x multiwell plate (96, 384) with a three-tube position loading cart

Maximal working volume:

- X : 220 mm / 8,66 inches
- Y : 155 mm / 5,91 inches
- Z : 50 mm / 1,97 inches

9.3. Power Supply and Connectors

Meanwell, GSM160A12-R7B, 100 - 240 V input to 12 V, 138 W certified IEC60601

Type	Specs
DC Power supply (Toby DIN 4p)	12 V, 138 W
USB for LUTHOR single cell dispenser control	2x USB

10. Appendix F: Warranty and Liability

The warranty for all apparatus and parts supplied by Lexogen lasts 24 months from the delivery date. If manufacturing or material faults become apparent within this period, Lexogen may choose a free replacement, repair, or credit entry. Lexogen is at liberty to carry out this work at the place of assembly or require the carriage-free return of the defective parts. Defects caused by incorrect handling, exceptional stresses, failure to follow the instructions for assembly, operating, maintenance, or unauthorized operations are not covered by the warranty. Equally not covered by the warranty is reasonable wear and tear of specified parts, degradation of batteries, and all glass components. Lexogen's warranty services apply only to the apparatus and parts supplied by Lexogen. Lexogen is not liable for any consequential damages incurred by the Renter. Support over and above the warranty service is charged at the currently applicable hourly rate of Lexogen.

11. Appendix G: Revision History

Publication No. / Revision Date	Change	Page
231UG794V0100 Sep. 16, 2024	Initial release.	

Associated Products:

204 (LUTHOR High-Definition Single-Cell 3' mRNA-Seq Kit)

205 (LUTHOR High-Definition Pool Single-Cell 3' mRNA-Seq Library Prep Kit)

221 (LUTHOR High-Definition Single-Cell 3' mRNA-Seq Kit with UDI 12 nt Set B1)

231.04 (LUTHOR single cell dispensing Kit)

LUTHOR single cell dispenser and single cell dispensing Kit · User Guide

Lexogen GmbH

Campus Vienna Biocenter 5

1030 Vienna, Austria

Telephone: +43 (0) 1 345 1212-41

Fax: +43 (0) 1 345 1212-99

E-mail: support@lexogen.com

© Lexogen GmbH, 2024

Lexogen, Inc.

51 Autumn Pond Park

Greenland, NH 03840, USA

Telephone: +1-603-431-4300

Fax: +1-603-431-4333

www.lexogen.com