DOTS
a platform by sbi

LIS user guide

A handbook for automated feeding in shake flask applications

sbi SCIENTIFIC BIOPROCESSING


DOTS
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Table of contents

Introduction and general considerations

Welcome to the LIS user guide and congratulations for your decision of choosing the DOTS platform and Liquid Injection System (LIS) to feed your shake flasks. This user guide will give you access to all information required to install and use the LIS within your laboratory everyday life. It is strongly recommended to read this user guide prior to any installation or operation works with the LIS.

This kind of grey box will be used throughout the document to indicate important aspects, hints, or summaries.

Caveats are indicated by yellow warning signs.

Dangers and risks are indicated by red danger signs.

In order to ensure that this user guide provides you all information you need during your work with the LIS, we at SBI are reliant on your feedback. Do not hesitate to contact us to share your ideas regarding errors, missing information, or incomprehensibilities so that we can improve this document and keep it up to date with your requirements.

This user guide is under continuous development and should be recognized as preliminary. This user guide, the DOTS platform and the LIS devices may be subject to changes and improvements without further notice.

In case of any question that might arise during the work with LIS and DOTS Software, do not hesitate to contact us. (Contact details on pp. 58).

LIS user guide revisions:

Revision 0	02.09.2022	- Initial document release

The LIS – an overview

LIS stands for **L**iquid **I**njection **S**ystem and enables automated feeding in shake flasks. The LIS is part of the DOTS platform and consists of the LIS Cartridges, LIS Drive, a Mounting System, the LIS Wireless Hub, and the DOTS Software.

Figure 1: The assembled LIS – Automated feeding for shake flasks.

LIS Cartridges

The LIS Cartridges, sterile filters, and Luer plugs are pre-sterilized, ready-to-use, single-use components. Once assembled with the LIS Drive, the components form a sterile chamber that holds the liquid to be dispensed into the shake flask.

Figure 2: The LIS Cartridge, filter, and Luer plug.

LIS Drive and Mounting System

The LIS Drive is the physical heart of the LIS-system, as it performs the communication and dispensing tasks. The Mounting System physically connects the LIS Drive and the Cartridge with each other.

Basic functions of the LIS drive can be controlled via the knob and display of the LIS drive. Also, basic standalone experiments are possible. For convenient control and full functionalities of the LIS, the LIS Drives are wirelessly connected to the DOTS Software via the LIS Wireless Hub (Figure 4).

Figure 3: The LIS Drive (left) and Mounting System, consisting of Octagrab (middle) and ring (right).

LIS Wireless Hub

The Wireless Hub handles communication between the DOTS Software and the LIS Drives. The hub connects to the computer by USB and to the LIS Drives wirelessly using the 2.4 GHz band.

Figure 4: The LIS Wireless Hub.

DOTS Software

The DOTS Software performs visualization, data analysis, post processing, exporting, and controlling, to provide LIS users with an intuitive solution to watch, analyze and store their experimental data. This user guide contains all information on the LIS hardware. The control of LIS hardware by the DOTS Software is documented in the DOTS Software User Guide.

LIS Flasks

We offer special LIS Flasks for LIS experiments (Figure 5). They contain one additional neck on the side, which can be sealed with standard Erlenmeyer flask caps. These side ports allow for sampling during an experiment without the need to remove the LIS, which mounts onto the top port.

Figure 5: LIS Flasks are available in standard shake flask sizes with an additional option for a third neck (second side opening).

Technical Specifications – LIS Drive

Input voltage	5 VDC ± 5%
Height (without Mounting System)	approx. 74 mm
Additional height on shake flask	approx. 70 mm
Temperature	
Operating temperature	15 – 45 °C
Charging temperature	15 – 40 °C
Battery (built-in)	
Type	Li-Ion
Capacity	600 mAh
Runtime when fully charged[1]	approx. 1 week
Charging time	approx. 3 h
Charging source	Standard USB port that can source 500mA @ 5V
Radio	
Frequency	2.4 GHz
Range[2]	up to 30 m
Pump flow rate	max. 1 mL/min
Cartridge	
Sterilization	Ethylene Oxide (EtO)
Required flask type	Straight neck (no brim)
Required flask outside diameter	38 mm (± 0.3 mm)
Minimum flask inside diameter	33 mm

1 Dependent on feeding profile

2 Dependent on environment

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Recommended operating conditions

Temperature	10 – 45 °C
Humidity (relative)	0 – 80% (non-condensing)
Filling volume	
Recommended	3 – 20 mL
Maximum[1]	25 mL
Maximum shaking speeds[2, 3]	
Shaking diameter ≤ 2.5 cm	
100 – 125 mL shake flasks	250 rpm
250 – 300 mL shake flasks	300 rpm
500 – 2000 mL shake flasks	250 rpm
Shaking diameter ≥ 5.0 cm	
100 – 300 mL shake flasks	250 rpm
500 – 1000 mL shake flasks	225 rpm
2000 mL shake flasks	200 rpm

1 Dependent on shaking speed and diameter

2 Tested with factory new Infors/Lauber spring clamps (full spring strength, not warped)

2 Values may differ when sticky mats or other adhesive mats are used or other addons are used (e.g., the Cell Growth Quantifier CGQ by SBI). Please carefully determine acceptable shaking speeds with your setup.

Warnings

Do not use the LIS or any of its components in water bath shakers! This could result in electric shocks, which could damage your health, the LIS, and other electric devices.

Be careful when mounting the LIS cartridge onto a flask. Do not try to force the cartridge onto a flask without a 38 mm neck as the flask could break.

Do not spill liquids over the LIS Drive.

Do not operate the system with flasks filled and/or operated in a way they can spill. Test each process condition before placing a flask on a sensor.

Avoid splashing of the liquid inside the flask (culture broth). Splashing flask liquid might reach the tip of the Cartridge, which could lead to contamination of your feed liquid.

Do not use the system in a way that any of its components might get damaged. If you are using process conditions that are far away from the recommended settings in this user guide, test the conditions beforehand and/or consult our Application Scientists. Any damage caused from wrong usage, known or unknown, excludes the system from our warranty

In general, do not use acids and bases, organic solvents, or detergents to clean the LIS Drive. Some organic solvents or detergents might be allowed for cleaning, but you should only use those mentioned in the user guide.

High shaking speeds, shaking throws, or the usage of highly volatile feed liquids might require other parameters or components to set up an experiment. If in doubt, consult our Application Scientists.

Always stop the shaker before doing anything with the LIS components or the shake flasks. Especially during assembly, the shaking movement should be turned off to avoid damage to your health and the devices and flasks on the shaker.

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Any kind of opening, manipulating, or copying of LIS devices, as well as decompiling, reverse-engineering, copying, or distributing of the DOTS software or the LIS firmware is strictly prohibited in accordance with German and international law and may lead to compensation claims.

SBI sensors, devices and other equipment are not intended for medical or military purposes or any other safety-critical applications. It is strictly prohibited to use SBI sensors, devices and other equipment for applications in humans or for applications where sensors are brought in direct contact with foods, drinks, tissues or other goods that are transferred into humans.

The LIS Drive contains a lithium-ion battery. Do not dispose the battery or LIS Drive into normal waste.

Do not heat or dispose of the battery in fire, water, or other liquids. Do not put the Drive into a microwave, washing machine, or a drying machine. Never use a damaged battery.

Never disassemble a LIS Drive. If a battery is crushed and the contents spill, use rubber gloves to handle all battery components. Avoid inhalation of any vapors related to the spill.

Take care when shipping the LIS Drive. Confirm shipping regulations for devices with lithium-ion batteries.

Do not charge or discharge the battery outside the operating temperature range (10-45°C).

For longer storage of the LIS Drive, the battery should be in a 50% charged state.

Declarations and Certificates

CE and UK conformity

The aquila biolabs GmbH, Arnold-Sommerfeld-Ring 2, 52499 Baesweiler, Germany, herewith declares under its sole responsibility that all devices and equipment being part of the LIS system and being manufactured by the aquila biolabs GmbH are in conformity with the Council Directives as described in EN IEC 61000-6-2:2019, EN IEC 61000-6-4:2019, EN 61326-1:2013, EN 55016-2-3:2017 + A1:2019, EN 61000-4-2:2009, EN IEC 61000-4-3:2020-09, EN 61000-4-4:2012, EN 61000-4-5:2014 + A1:2017, EN 61000-4-6:2014, EN 62368-1:2014+A11:2017, EN62479:2010, EN 301 489-1, EN 301 489-17, EN 300 328.

This declaration applies to all products with the following identifiers:

- LIS-LISO

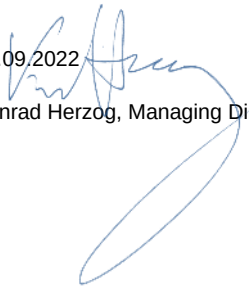
- LIS-DRIVE

Technical documentation is maintained at the aquila biolabs GmbH headquarter in Arnold-Sommerfeld-Ring 2, 52499 Baesweiler, Germany.

Date of declaration: 01.09.2022

Name, position of the undersigned: Konrad Herzog, Managing Director

Research & Development



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WEEE conformity

WEEE-Registration-No.: 61144888

The aquila biolabs GmbH, Arnold-Sommerfeld-Ring 2, 52499 Baesweiler, Germany, herewith declares compliance of all electronic components of the LIS system with the Council Directive 2012/19/EU.

Electronic components may contain various hazardous substances that could possibly exhibit negative impacts on your health and the environment. In order to avoid those effects aquila biolabs encourages you to make use of the appropriate local take-back and recycling systems for disposing electrical and electronic equipment. By doing this you are furthermore significantly reducing the pressure on natural resources and thus preserve our planet for subsequent generations.

Components that are affected by this declaration carry the following pictogram:

Date of declaration: 01.09.2022

Name, position of the undersigned: Konrad Herzog, Managing Director

Research & Development

FCC compliance

Contains FCC-ID: VNR-ATSAMR210UA-0

This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions:

(1) this device may not cause harmful interference, and

(2) this device must accept any interference received, including interference that may cause undesired operation.

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

Industry Canada (IC) compliance

Contains Transmitter Module IC: 20266-ATSAMR210UA0

This device complies with Industry Canada license-exempt RSS standard(s). Operation is subject to the following two conditions:

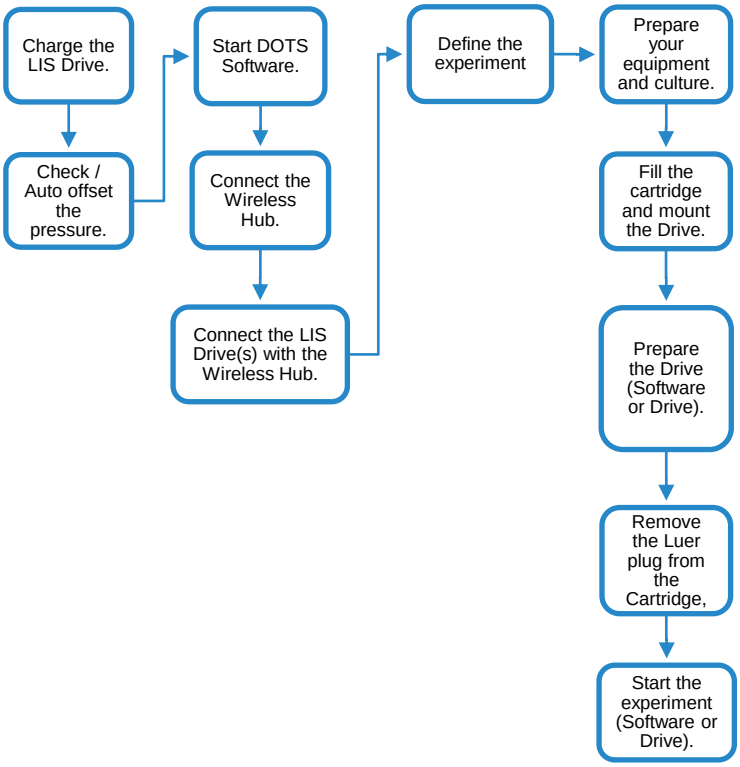
(1) this device may not cause interference, and

(2) this device must accept any interference, including interference that may cause undesired operation of the device.

This equipment complies with radio frequency exposure limits set forth by Industry Canada for an uncontrolled environment. This equipment should be installed and operated with minimum distance 20 cm between the device and the user or bystanders.

Quick start Checklist

The quick start checklists summarize the steps required to start an experiment. The descriptions how to conduct the individual steps are written in chapter LIS setup and handling.



LIS setup and handling

LIS setup – an overview

The workflow to set up an experiment with the LIS is as follows:

1. Charge the LIS Drives.
2. Perform “Auto offset pressure” on the LIS Drives.
3. Connect LIS Drives to DOTS Software via a LIS Wireless Hub.
4. Configure the LIS Drive via DOTS Software.
5. Assemble the LIS Drive/Cartridge assembly on top of the experiment flask:
    - Fill the Cartridge and assemble the LIS Drive/Cartridge on top of a flask.
    - Perform “Prepare” on the LIS Drive.
6. Start the experiment via DOTS Software.

The individual setup steps for the hardware are explained in detail below. Information on all workflows of the DOTS Software can be found in the DOTS Software User Guide.

Charging the LIS Drive

Always use fully charged LIS Drives for your experiments. The battery usage depends on your setup, i.e., how intense the pump of the LIS Drive is working. Influencing factors are, amongst others, the programmed feeding profile, the type of liquid, surrounding temperature (changes), and shaking speed.

To charge a LIS Drive, connect the micro-USB charge port (Figure 6) to a free USB port (computer or wall-plug charger) using a micro-USB cable. It will take approximately 3 hours to fully charge a LIS Drive.

Unplug the charger after charging is complete. Do not let the LIS Drive charge for longer than four hours.

Figure 6: A powered-on LIS Drive with an arrow indicating the micro-USB charge port .

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Auto Offsetting the pressure on the LIS Drive

Before using the LIS Drive, the pressure sensor needs to be checked and reset. The Drive has a function called "**AutoOffset**" that sets the current measurement to zero.

Make sure no filter or cartridge is connected to the Drive before offsetting the pressure.

Navigate through the menu of the LIS Drive by turning and pressing the knob on top of the Drive (see section "Controlling the LIS Drive via the Drive Menu" pp. 38, Press=Enter).

1. Go to **"Settings**" then **"Pressure"** and check if the value of the entry **"Pressure"** is around 0 (± 5 counts).
2. If the value differs, go to **"AutoOffset"** and press the knob to confirm.
3. Now the Pressure is reset to zero and the LIS Drive is ready to use.

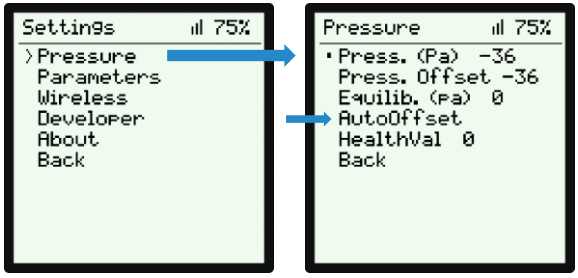

Figure 7: Using the AutoOffset feature on the LIS Drive menu to set the pressure measurement to zero.

The LIS Drive should be checked for correct dispensing on a regular basis. Contact our support team to learn how and to get instructions on Drive calibration, if required.

Connecting the LIS to the DOTS Software

The latest firmware should be installed on the LIS Wireless Hub and the LIS Drive. Contact our support team for instructions on how to update devices.

Connect the **LIS Wireless Hub** to the computer with the DOTS Software installed using the supplied micro-USB cable. The green LED of the LIS Wireless Hub should light up. The LED blinking patterns for the Wireless Hub are explained on page 57.

Open the DOTS Software in your browser. Navigate to the “Devices” tab and check the PanID (one or two-digit number) of the connected Wireless Hub. If the PanID of the new Wireless Hub is the same as another Wireless Hub that is already integrated in the DOTS Software, contact your DOTS Software administrator to have one of the PanIDs updated to a new value.

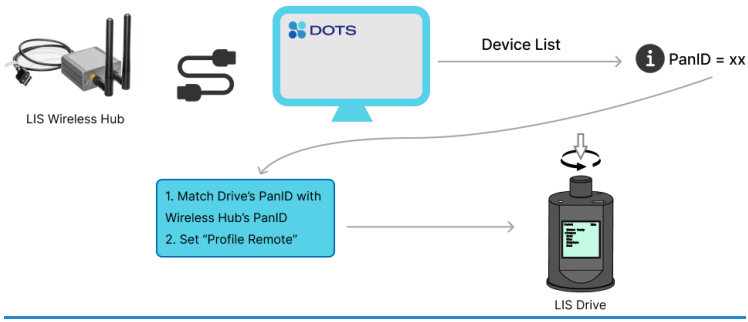

Figure 8: Connecting the LIS to the DOTS Software.

For detailed explanations on
- **How to control the LIS Drive manually**
- **The Drive menu structure and functions**

Refer to section “LIS standalone experiments” on page 48.

Configuring the LIS Drive connection to the Wireless Hub

The **LIS Drive(s)** will connect to the Wireless Hub if the LIS Drive and Wireless Hub share the same PanID. If this has not been configured, follow these steps:

1. Power on the LIS Drive (press the knob for > 1 sec).
2. Navigate through the LIS Drive menu by turning and pressing the knob (Press=Enter).
3. In the LIS Drive menu, go to **"Settings"**, then **"Wireless"**.
4. Configure the number behind "**PanID**" to match the PanID of the Wireless Hub. Press the knob to confirm.
5. If the PanID was entered correctly but the LIS Drive is not connected to the software, press the **"Reconnect"** button at the LIS Drives **"Wireless"** Menu.

Allow the Drive to search for the Wireless Hub for at least one minute before reconnecting it.

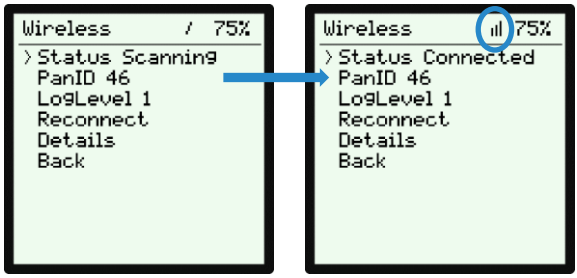

Figure 9: Updating the PanID on the LIS Drive menu. Once the LIS Drive is connected an indicator is shown in the top right corner of the display.

Configuring the LIS Drive(s) for DOTS Software control

In the LIS Drive menu, go to “Experiment” and change “**Profile**” to “**Profile Remote”**. Press the knob to confirm.

Figure 10: The LIS Drive menu setting for DOTS Software control.

Connecting multiple LIS Drives and Wireless Hubs

One Wireless Hub can handle up to 32 LIS Drives and multiple Wireless Hubs can be connected to the same controlling computer. In this scenario each Wireless Hub should have a unique **PanID** and **Channel** setting. Couple an even amount of LIS Drives to each Hub by setting the Drive PanIDs to the corresponding Hub PanID value.

Configuring the LIS Drive(s) in the DOTS Software

The process for how to create and start an experiment in the DOTS Software is explained in detail in the DOTS Software User Guide. The high-level steps are listed below.

1. Click the button to create an Experiment and select a suitable Application Template for your Object of interest.
2. Configure your LIS feeding profile and other monitoring Tasks.
3. Assign a device to each Object.
4. Create your Experiment.
5. Perform the Task Action “Upload LIS” to send your configuration to the LIS Drive(s) and start the LIS Workflow.

Assembling and preparing the LIS

Fill the Cartridge and assemble the LIS on top of a flask

Before you start, make sure you have prepared everything you need to set up a LIS experiment.

LIS components

- A LIS Wireless Hub connected to a PC with the DOTS Software installed
- One LIS Drive per flask
  - Fully charged battery
  - Connected to the LIS Wireless Hub
  - Pressure auto offset
  - Configuration uploaded to the Drive by DOTS Software
- One mounting system per flask
- One LIS Cartridge including sterile filter and Luer plug per flask

From your lab

- All required (LIS) flasks, filled with medium and inoculated
- At least one additional waste vessel
- The liquid to be fed
- Pipettes, tips, measuring cylinders, gloves, if required

The LIS Cartridge is designed to fit 38 mm (± 0.3) straight neck Erlenmeyer shake flasks. Do not try to fit the cartridges on other types of flasks.

Figure 11 shows a process overview of the assembly of the LIS (cartridge with feeding liquid, Drive and Mounting System (Octagrab+Ring). The individual process steps are explained in detail below the graphic.

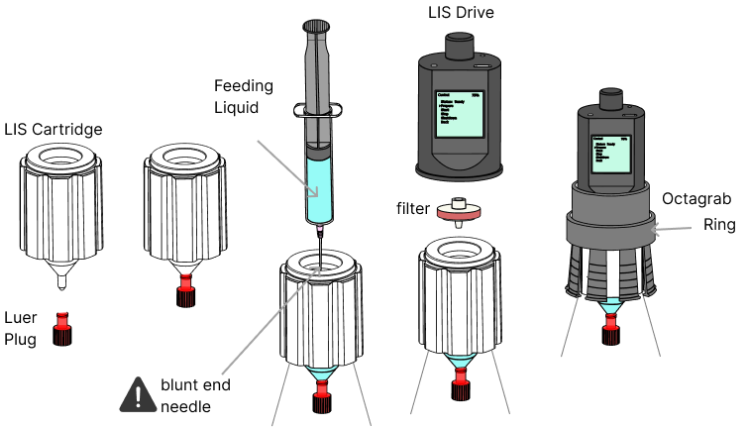

Figure 11: Process of assembling the LIS on top of a flask and filling the LIS Cartridge with feeding liquid.

Place your sterile flask in a sterile environment.

Open a pre-sterilized LIS Cartridge and the sterile Luer plug package. Use the Luer plug to close the cartridge (Figure 11, step 1).

Put the sealed cartridge on the flask.

Figure 12: Assembling the LIS – Preparing the sterile Cartridge.

Attention: The LIS Cartridge is a single-use product. Proper performance can only be guaranteed for one-time usage. Repeated use will lead to leakage.

LIS Cartridges are not autoclavable.

With a Syringe and a needle (recommended: blunt end needle), slowly fill the cartridge “from the bottom”, i.e., with the needle fully inserted to prevent splashing.

Insert the needle through the small Luer opening in the lid of the cartridge.

Make sure to fill with no more than 25 mL of liquid and record how much volume is in the cartridge.

Ensure no liquid is on the lid after you fill the cartridge (this could wet, i.e., block the filter in the next step).

Figure 13: Assembling the LIS – Filling the Cartridge.

Install the sterile filter on the lid of the cartridge. Push the filter gently into the hole. Applying too much pressure might deform the lid or hole, resulting in a leaky Drive-Cartridge-Assembly.

Figure 14: Assembling the LIS – Installing the sterile filter on the Cartridge.

Make sure the LIS Drive is fully charged and you have auto offset the pressure as described on page 22.

Install the LIS Drive on the cartridge by placing it on the sterile filter. Do not apply pressure on the LIS Drive as this might damage the LIS Drive.

Figure 15: Assembling the LIS – Placing the Drive on the Cartridge.

Use the Octagrab and move it from the top, down over the LIS Drive. Make sure the arms of the Octagrab reach over the bulges of the cartridge.

Figure 16: Assembling the LIS – Coupling the Drive with the Cartridge using the Octagrab.

Place the ring of the Mounting System over the LIS Drive and Octagrab and slide it down as far as it will easily go.

No need to tighten yet.

Make sure to slide the ring far enough down so the small arms at the end of the Octagrab are **ALL** underneath the cartridge.

Figure 17: Assembling the LIS – Securing the Drive and Cartridge together by placing the Mounting System ring over the Octagrab.

Perform "Prepare" on the LIS Drive and remove the Luer plug

Once the LIS Drive has been assembled on top of the filled cartridge, the internal pump must be turned on to adjust the pressure inside the cartridge. This step is called "Preparing the Drive" and it enables the Drive to hold the liquid inside the cartridge. Figure 18 shows the process of preparing the LIS for an experiment. The individual process steps are explained in detail below the graphic.

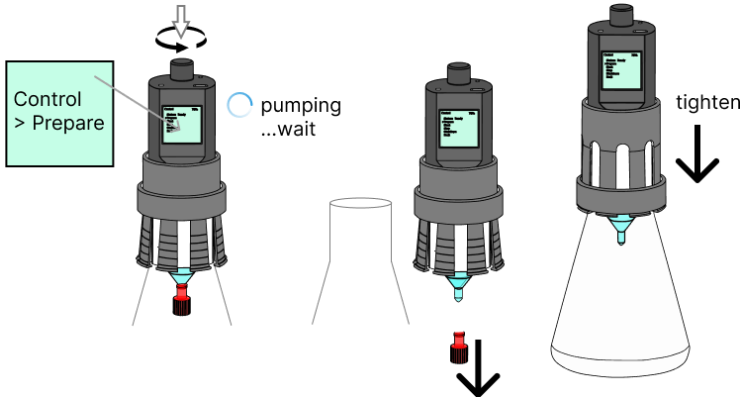

Figure 18: Process of Preparing the Drive of an assembled LIS.

Prepare the Drive. You can do this manually on the Drive or in the DOTS Software.

Manual: In the Drive menu, navigate to Control > Prepare. Press the knob to confirm.

In the DOTS Software: See the DOTS Software User Guide for detailed instructions

You will hear a noise as the Drive starts pumping. Wait for the pumping to become slower (it will almost, but not completely stop).

The Status of the Drive will change to “Prepared”

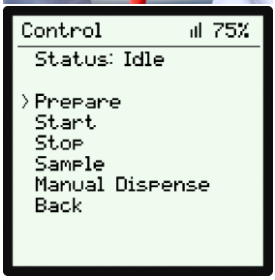

Figure 19: Preparing the LIS Drive – Manual process from the Drive menu.

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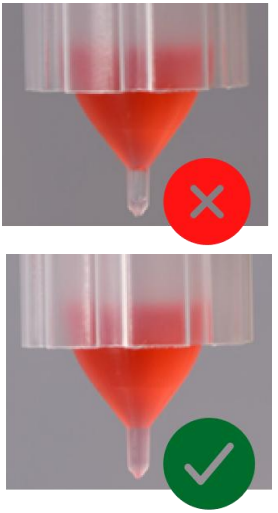
Carefully remove the whole LIS assembly from the flask.

Maintain the connection between the Drive and the Cartridge, making sure the Octagrab arms remain underneath the bottom rim of the Cartridge.

Hold the assembly over a waste vessel and remove the Luer plug.

Do not tilt the assembly! The sterile filter inside must not get wet.

Figure 20: Preparing the LIS Drive – Removing the Luer plug.



Now, the liquid should stay inside the Cartridge. Make sure that the cartridge outlet is **completely filled** with liquid and no air bubble is present. If there is an air bubble, manually dispense a few µl as explained in the section “Using a needle with your LIS”.

Refer to page 40 for how to navigate to the “Dispense” function on the LIS Drive display.

Figure 21: Preparing the LIS Drive – Ensuring no air bubble is trapped in the Cartridge outlet.

Put the LIS assembly back on the flask and carefully tighten the assembly by pushing the ring of the mounting system down over the Octagrab.

The hooks at the end of the Octagrab's arms must reach underneath the bulges of the Cartridge.

Since the Cartridge is now open, the system is prone to hits from the top as this might result in unwanted dripping.

To avoid this, "screw" the assembly back to the flask by using twisting motions instead of pushing.

.

Do not squeeze the cartridge from the sides, or liquid might spill into your flask.

Figure 22: Preparing the LIS Drive – Replacing the LIS back onto the flask after removing the Luer plug.

The LIS Cartridge is designed to fit 38 mm straight neck Erlenmeyer shake flasks. Do not force the cartridge onto flasks with larger or smaller outer diameter as the glass could break.

The LIS is now assembled.

- If no needle is used (most applications), follow the steps in section "Starting a LIS experiment" to start an automated feeding process.
- If a needle is used, follow the additional steps described below.

Using a needle with your LIS

This is an optional step. The usage of a needle to lengthen the cartridge outlet is recommended for certain applications (i.e., when using shake flasks with a volume of 500 mL or greater) to ensure that drops of liquid fall into the culture instead of hitting the side of the flask.

To add a needle, take your previously assembled LIS **BEFORE** the Drive has been prepared and perform the following steps.

1. Holding the assembled LIS over a waste container, remove the Luer plug on the Cartridge outlet. This is where the needle will attach.

A few drops of liquid may leave the Cartridge since the chamber has not been pressurized.

2. On the Drive menu, go to **"Control"**, **"Manual Dispense"** and move the arrow to **"Dispense"**. DO NOT CLICK!

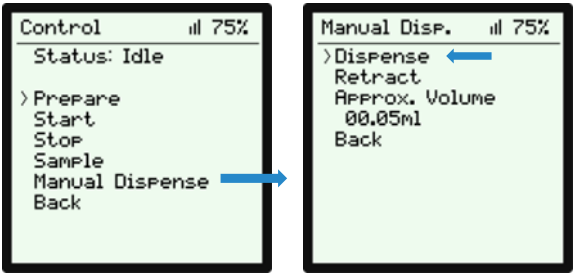


Figure 23: Navigating to the "Dispense" function on the Drive menu.

3. Take a sterile needle (e.g, a B. Braun Sterican needle) and hold it at a 45° angle under the Cartridge outlet.
4. Press the knob of the Drive to dispense some µL of the liquid. Repeat this step until the plug of the needle is filled with liquid.

Make sure the liquid flows into the female connector portion of the needle and displaces the air - do this step without connecting the needle completely to the Cartridge tip.

Figure 24: Filling the headspace of the sterile needle with liquid.

5. Attach the needle to the cartridge and press **"Dispense"** until liquid starts to flow out of the needle. The needle is now primed.
6. Follow the steps starting on pp. 30 to "Prepare" the Drive with the needle attached. Disregard steps related to removing the Luer plug, as this has already been done when priming the needle.
7. Once the Drive is prepared, the LIS Drive with needle can be transferred to the experiment flask.

It is important that no air bubbles are present in the needle plug after the priming step. Refer to Figure 25 - Figure 27 for examples of what to avoid and how a correctly primed and connected needle should look.

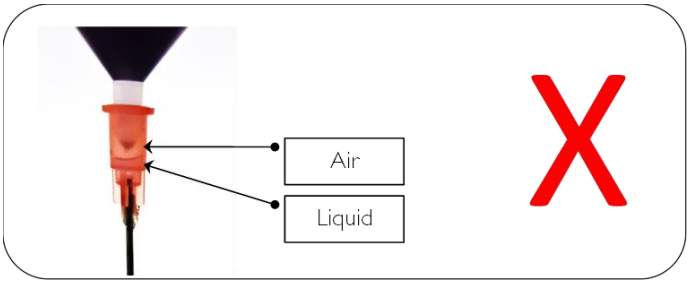


Figure 25: Air between the Cartridge outlet and the needle.

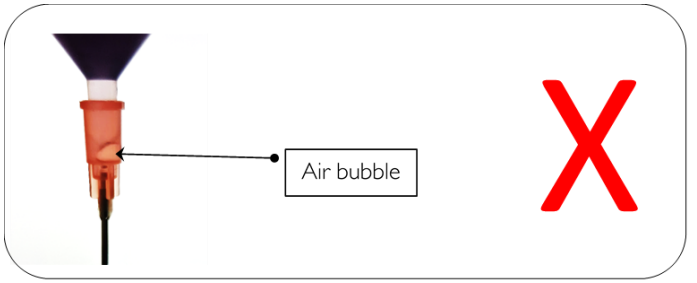


Figure 26: Air bubble stuck on the Cartridge outlet.

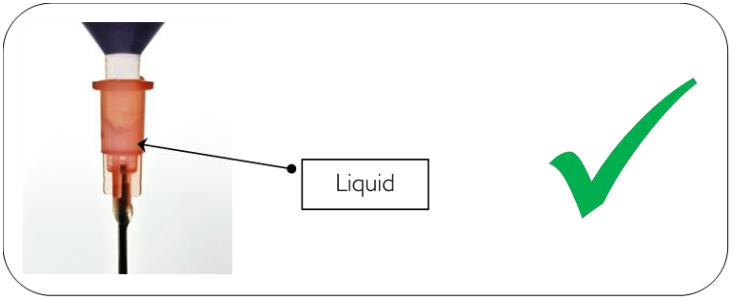


Figure 27: Correctly primed needle with no air in the needle's headspace.

Adding a needle to the LIS will increase the height of the water column. To avoid unwanted drop release, the LIS Drive pressure parameters need to be adjusted. Contact our support team for information regarding the right parameters for your application.

Starting a LIS experiment

Once assembled and prepared (with or without a needle) the LIS can be used in an experiment. Carefully mount the Flask-LIS assembly in your shaker.

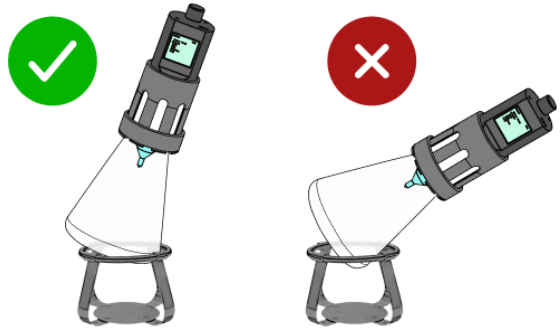


Figure 28: Placing a Flask-LIS assembly in a spring clamp.

Avoid tilting the filled cartridge too much. The liquid inside the cartridge must not come in contact with the sterile filter as this will block the filter.

Once the system is in "Prepared", or "Started" mode, it holds the liquid inside the Cartridge by maintaining a negative pressure. However, strong shocks should be avoided as they could lead to unwanted drop release into the shake flask.

The process for how to create and start an experiment in the DOTS Software is explained in detail in the DOTS Software User Guide. The high-level steps are listed below.

1. Click the button to create an Experiment and select a suitable Application Template for your Object of interest.
2. Configure your feeding tasks and assign a device to each Object
3. Create your Experiment.

Controlling the LIS Drive via the Drive Menu

Manual control of the LIS Drive

Although most functions are controlled via the DOTS Software, there are certain steps in an experiment that require manual control of the LIS Drive.

- Turning the Drive on and off
- Establishing a new connection between a LIS Drive and a LIS Wireless Hub (set a PanID).

Manual control is also recommended for the following functions.

- Auto-offset the pressure
- Initiate “Prepare” step prior to an experiment with the LIS Drive

The LIS Drive can also operate independently from the DOTS Software, but these experiments lack several functions such as multistep feeding profiles (for example: delay → single shot → continuous feeding), the exponential feeding profile, and external feeding triggers such as biomass. Hence, using the LIS as a standalone device is not recommended.

This chapter will introduce the functions available on the LIS Drive screen and explain how the LIS Drive can be controlled manually via the Drive menus. A flow chart with an overview of the LIS Drive menu structure is included at the end of the chapter.

Knob functions

Pressing and holding the knob for approximately 1 second turns on the LIS Drive.

Twisting the knob left or right allows you to move in the menu and/or increase and decrease numerical values.

- Values that can be altered or menu entries that can be selected (i.e., comprise a sub-level) are marked with a “>” cursor.
- Values that cannot be modified are marked with a “ ” cursor

A Single press on the knob selects an entry or confirms an input.

Pressing and holding the knob for more than 5 seconds triggers a forced shutdown of the LIS Drive (only use this function if problems occur).

Figure 29: Knob functions.

Main menu display

- **Status:** Status of the Drive.
- **Control:** Start or stop an experiment.
- **Experiment:** Setup an experiment.
- **Settings:** Change settings of the LIS Drive.
- **Shutdown:** Power off your LIS Drive.

Figure 30: Main menu functions.

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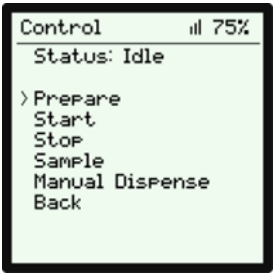
Status menu display

- **Status:** Status of the Drive.
- **Elapsed Time:** Time since the experiment started.
- **Remaining Time:** Time until the experiment is finished.
- **Dispensed Volume:** Total volume that has been dispensed.
- **Remaining Volume:** Volume still to be dispensed.

Figure 31: Status menu functions.

Control menu display



- **Idle status:** No experiment running or experiment finished.
- **Run status:** Pump is busy during an in-progress experiment.
- **Prepare status:** Pump is equilibrating pressure to hold liquid inside cartridge.
- **Prepared status:** Prepare function was successful and experiment can be started.

- **Prepare:** Equilibrate pressure to hold liquid inside cartridge
- **Start:** Start a *standalone* experiment, and the clock on a prepared experiment.
- **Stop:** Stop a *standalone* experiment.
- **Sample:** Suspend feeding for manual sampling during a *standalone* experiment.
- **Manual Dispense**: Manually dispense liquid from the Cartridge.
  - **Dispense:** Dispenses the approximate volume.
  - **Retract:** Retracts the approximate volume.
  - **Approx. Volume:** Volume to be dispensed/retracted.

Figure 32: Control menu functions.

Experiment menu display

This menu includes the experiment profiles which control the LIS Drive pump. In general, the LIS Drive is controlled via the DOTS Software (set “Profile Remote” to allow control by the Software). For standalone experiments, feeding profiles can be set on the Drive. Each profile can be used to create a unique feeding pattern. See section “LIS Drive feeding profiles” for more information.

- **Profile Remote:** Dispensing is controlled by the DOTS Software. In this case, no other options are available on the LIS Drive.
- **Profile Single/Multi/Constant** Dispensing is controlled on the Drive itself. These profiles are for standalone experiments, only.
- **Repetitions (only “Profile Multi”):** Number of dispensing steps to discharge the dispense volume.
- **Dispense Volume:** Overall volume to be dispensed into the flask.
- **Filling Volume:** Volume of liquid in the cartridge at the beginning of the experiment.
- **Initial Delay:** Time before first dispensing action.
- **Runtime (not in “Profile Single”):** Total time of the experiment including any initial delay and the dispensing time. The dispensing time is calculated based on a pump speed of approximately 1 mL/min and will be auto-adjusted.

Figure 33: Experiment menu functions.

Settings menu display

- **Pressure:** Inspect and edit pressure parameters.
- **Parameters:** Inspect and edit experiment-specific parameter sets.
- **Wireless:** Inspect and edit the wireless connection.
- **Developer:** Only used during production.
- **About:** Drive and Firmware information

Figure 34: Settings menu functions.

Pressure settings display

- **Press. (Pa):** Current differential pressure value in Pascals, minus any offset
- **Press. Offset:** Stored offset value. Can be set using 'AutoOffset' function.
- **Equilib. (Pa):** Target value of the pressure controller. Should be close to the current differential pressure. Only valid with active experiment.
- **AutoOffset:** Sets Press. (Pa) to zero. Only use auto offset while no filter/cartridge is attached to the Drive.
- **Health value:** Measures the experiment health in arbitrary units. Smaller values are better.

Figure 35: Pressure settings menu.

Parameter settings display

- **Pulse/10 uL:** The number of pump cycles required to dispense 10 µL of liquid from the Cartridge. This value is factory calibrated.
- **P1-P8:** Parameters for the pressure-control algorithm.

Figure 36: Parameter settings menu.

Wireless settings and Details display

- **Status:** Connection status.
- **PanID:** Like a WiFi network name, the Drive PanID must match the Hub PanID.
- **LogLevel:** Used for debugging. Values ≠ 0 will make debugging messages appear in the communication log-file.
- **Reconnect:** Restart the wireless connection.
- **Details:** Connection details
- **Link Quality:** Values are between 0 and 255. Note, this is a non-linear rating.
- **Channel:** 802.15.4 radio communication channel (frequency). Can be changed at the Wireless Hub.
- **Antenna:** Indicates which type of antenna is currently used.
- **Frame Count:** Number of received data packets.
- **IEEE MAC Address:** Drives' individual MAC Address.

Figure 37: Wireless settings and Details menus.

Developer settings display

- **Acceleration:** Displays acceleration data, can be used to verify sensor functionality.
- **Enter Boot:** Enters boot mode to enable firmware updates.
- **Do Crash:** Test the recovery of experiments.
- **Testmode:** Various production tests.
- **Load Defaults:** Reset Drive settings to default values. This also overrides calibration values.
- **Recovery Count:** Number of times the firmware recovered.
- **Bat Volt:** Battery Voltage in mV.
- **Bat Curr:** Charge current in mA.

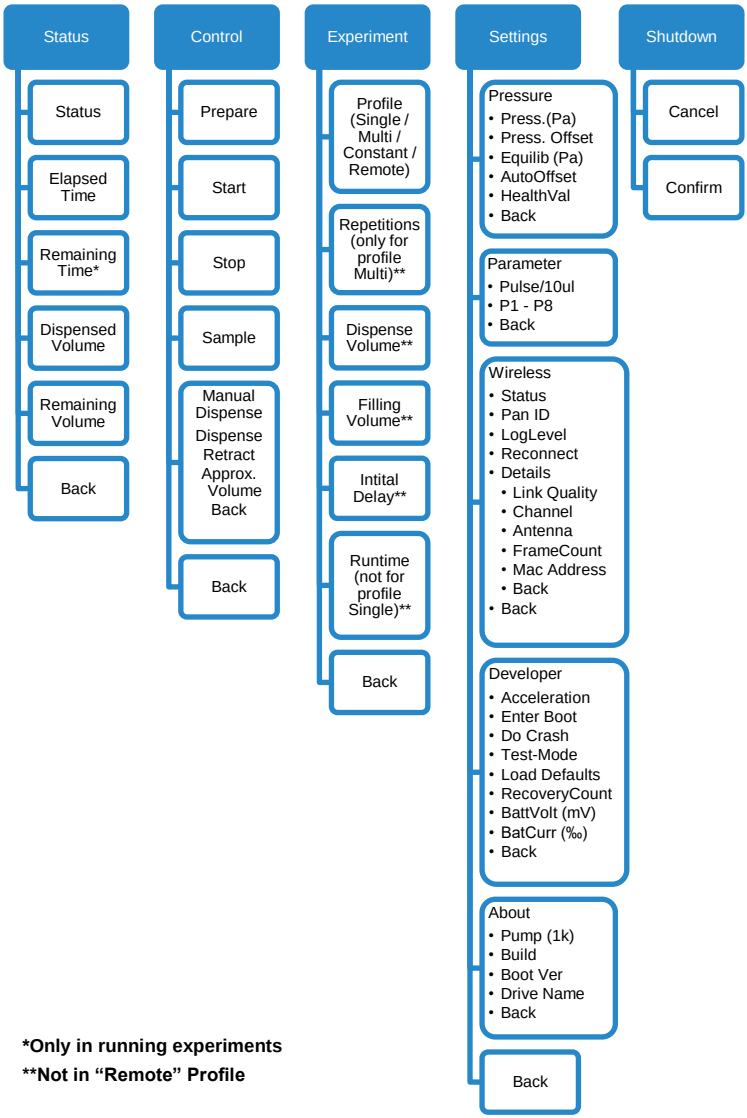
Figure 38: Developer settings menu.

About display

- **Pump (1k):** Pump Lifetime tracker.
- **Build:** Firmware version information.
- **Boot Ver:** Bootloader version information.
- **Drive Name:** User-specified name.

Figure 39: About menu.

LIS Drive menu structure overview



***Only in running experiments**

****Not in "Remote" Profile**

LIS Drive feeding profiles

There are three types of feeding profiles available for configuration on the LIS Drive Experiment menu (*for standalone experiments*). These, and many other options for feeding, are also available for configuration with the DOTS Software. Refer to the DOTS Software User Guide to learn more.

The **Single** feed profile causes the liquid in the Cartridge to be dispensed in one shot after a user-defined, initial delay (Figure 40).

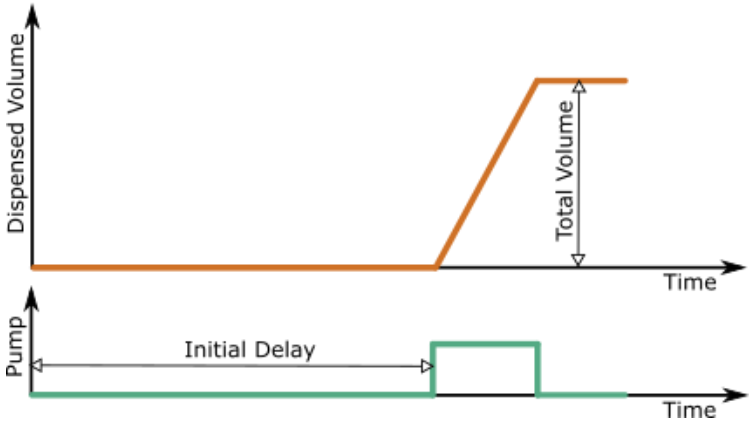


Figure 40: A single-shot feed profile with an initial delay, showing volume dispensed and pump activity over time.

The **Multi** feed profile causes the liquid in the Cartridge to be dispensed in a stepwise manner after the initial delay, with a user-defined number of feed steps. An even amount of liquid is dispensed at each step (Figure 41).

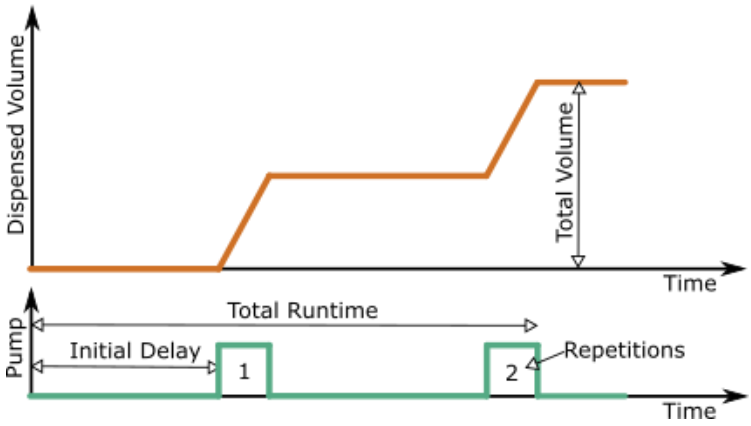


Figure 41: A multi-shot feed profile with an initial delay, showing volume dispensed and pump activity over time.

A **Constant** feed profile causes the liquid in the Cartridge to be dispensed evenly over a user-defined period after the initial delay.

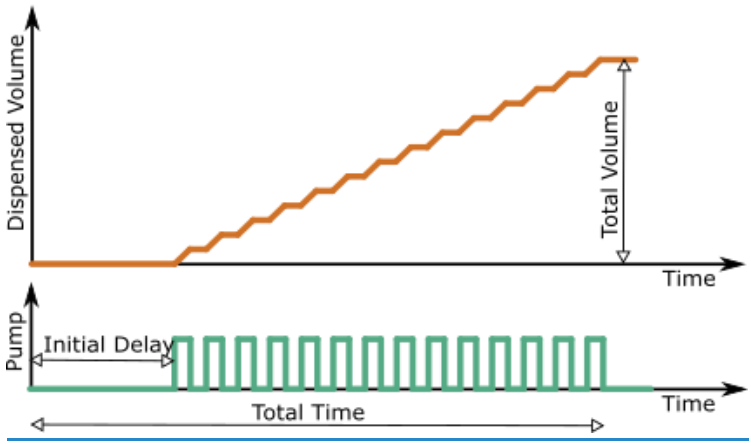


Figure 42: A Constant feed profile with an initial delay, showing volume dispensed and pump activity over time.

LIS standalone experiments

The LIS Drive can operate independently from the DOTS Software and the LIS Wireless Hub. Standalone experiments are limited to LIS-alone experiments meaning external feeding triggers are not possible. For extended functionalities and a more user-friendly control, use the LIS with the DOTS Software.

Set up and start a LIS standalone experiment

Once the pressure has been auto-offset, an experiment can be started on the LIS Drive.

1. Navigate to the "**Experiment**" menu on the Drive, choose one of the feeding profiles, and define the feeding parameters.
2. Check the pressure parameters ("**Settings**" → "**Parameters**") and update if necessary. Please contact our support team to verify if a new parameter set is required for your application.

Using the wrong parameter set might lead to inaccuracy, unwanted drop release, or no dispensing. Please contact our support team for more information regarding the right parameters for your application.

3. Fill the cartridge and assemble the LIS. Make sure to Prepare the Drive and remove the Luer plug from the Cartridge outlet.
4. Carefully place the assembled LIS on a flask.
5. Taking care not to tip the coupled flask-LIS too much, fix the flask on the shaker.
6. Start your Experiment.

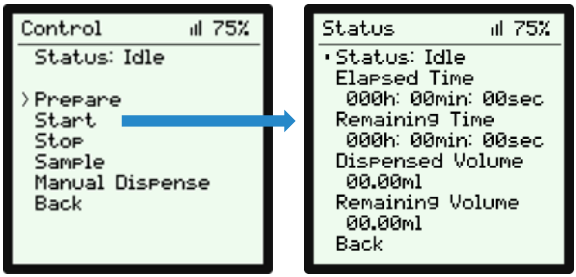


Figure 43: Starting a standalone LIS experiment from the "Control" menu on the Drive.

On successful start, you will be forwarded to the "Status" screen where you will have an overview of your experiment. As the experiment progresses, the elapsed time will increase and the remaining time decrease.

Avoid tilting the filled cartridge too much. The liquid inside the cartridge must not come in contact with the sterile filter as this will block the filter.

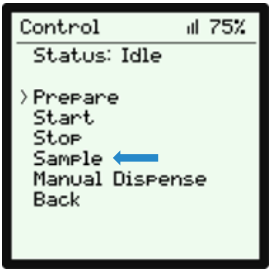
Avoid strong shocks as they could lead to unwanted drop release into the shake flask.

The LIS Cartridge is designed to fit 38 mm straight neck Erlenmeyer shake flasks. Do not force the cartridge onto flasks with larger or smaller outer diameter as the glass could break.

Pausing and stopping the LIS Drive

Pause the LIS Drive for sampling

If you want to take a sample from your culture or perform another manual task during an experiment, you can use the **"Sample"** function on the LIS Drive to pause the feeding process. All dispense actions are postponed until sampling mode is disabled and the LIS Drive resumes the feeding experiment.



Navigate to the "**Control**" menu on the Drive and select "**Sample**". The status should now say "**Sample**" and the green LED on the Drive will blink.

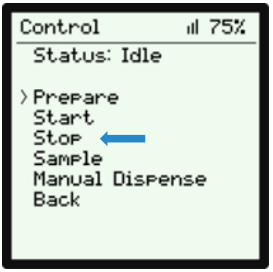
Remove the assembled LIS system from the flask and perform manual tasks.

Return the LIS to the top of the flask, again making sure to twist and not push the Cartridge down onto the flask.

Select "**Sample**" again to exit the sampling mode. The Drive status should now say "**Run**".

Figure 44: Select "Sample" from the "Control" menu to pause a LIS experiment.

Stop the LIS Drive



Navigate to the "**Control**" menu on the Drive and select "**Stop**".

To power off the Drive, navigate to the main menu and select "**Shutdown**".

Figure 45: Select "Stop" from the "Control" menu to stop a LIS experiment.

Pressure parameters

Information on the LIS parameter set

As the LIS maintains the pressure in the headspace of the LIS Cartridge, several physical properties need to be considered. For example, the height of the water column, the density of the liquid inside the cartridge, or the shaking speed.

All relevant properties are modeled into the parameter set. Since the parameter set is an abstraction, no direct correlation between physical values (density, …) and the actual parameter value exist.

The “Standard Parameters” supplied with the LIS Drive and DOTS Software are designed to work with water-like solutions, as long as no needle is attached to the LIS Cartridge. In addition to this standard parameter set, the DOTS software also holds parameter sets for several types of liquids and solutions that are frequently used in biological labs. Contact our support team if you need a custom parameter set for your application.

Disinfection

Information on cleaning the LIS

The LIS Drive and cables can be disinfected by wiping them softly with 70% ethanol wipes.

Ensure that you have disconnected all LIS devices and cables from any kind of power supply, to prevent damages to the electronics, to connected devices, and to your health.

Ensure that all LIS devices and cables are completely dry before you reconnect them to each other, to the power supply, and the USB.

Troubleshooting

Table of common problems and solutions

Refer to the table below for suggested causes and solutions to common problems seen with the LIS.

Leaky Cartridge / pump does not stop while preparing Drive

Possible causes:	Solutions:
Repeated use of cartridge.	The cartridge is a single use product. Use a new cartridge.
Wrong filling volume entered.	Input the correct filling volume under “Experiment” and hit prepare to check
Wrong pressure parameters.	The default parameters are for water and comparable solutions. If you have solutions with different physical properties (e.g., significantly higher / lower density) you need to adjust the parameters. Make sure to always use a parameter set that was designed for the liquid and process conditions (especially: temperature and shaker throw and speed). Using the wrong parameter set will lead to poor dispensing or dripping. Contact SBI if the required parameter set is not available in the DOTS software.
Drive is switched off.	Several reasons exist why this can happen. For example, safety (wet filter – prevent from over pressurizing) or wrong handling. See problem "Why does my Drive switch off" for details
Wrong pressure offset.	See section “Auto Offset the Pressure”

Air bubbles rising through the nozzle

Possible causes:	Solutions:
Wrong filling volume entered.	See above
Wrong pressure parameters.	See above
Luer plug removal.	Sometimes this just happens when removing the Luer plug. Replace the Luer plug, Prepare again, and remove the plug again.

Drive display shows “BOOT” / LED blinks red and green

Possible causes:	Solutions:
The Drive is in bootloader mode.	Long press (> 5 seconds) the knob to power off the Drive. After turning it on again it will be in normal operation mode.

Dispensing accuracy not acceptable

Possible causes:	Solutions:
Pressure or calibration parameters do not fit the liquid properties.	The default parameters are for water and comparable solutions. If you have solutions with different physical properties (e.g., significantly higher / lower density) you need to adjust the parameters. Make sure to always use a parameter set that was designed for the liquid and process conditions (especially: temperature and shaker throw and speed). Using the wrong parameter set will lead to poor dispensing or dripping. Contact SBI if the required parameter set is not available in the DOTS software.
Drive must be calibrated.	The Drive uses a peristaltic pump, and the tube characteristics might change over time. Hence, the Drive must be checked and calibrated regularly. Contact our support team to learn more.

Drive does not power on

Possible causes:	Solutions:
Battery empty.	Connect to a suitable charger.

Drive shut down during experiment

Possible causes:	Solutions:
Battery drained due to excessive pump activity.	See “Why does my drive switch off”
Low battery level at experiment start.	Start experiment with a fully charged Drive.

LIS Wireless Hub not shown in the DOTS Software

Possible causes:	Solutions:
Device has not been added to the DOTS software.	Ask your admin to connect the Device for the first time to the DOTS Software. Then ask your admin or Team leader to assign the device to your Team.

LIS Drive not shown in the DOTS Software

Possible causes:	Solutions:
Device has not been added to the DOTS software.	Ask your admin to connect the Device for the first time to the DOTS Software. Then ask your admin or Team leader to assign the device to your Team.
LIS Wireless Hub not properly connected.	Connect the LIS Wireless Hub to the DOTS Software.
LIS Drive turned off.	Turn on the LIS Drive
PanID incorrect, or Wireless deactivated on the LIS Drive.	On the LIS Drive, go to “Settings” → “Wireless” → “PanID” and set the correct PanID.
LIS Drive not connected properly	On the LIS Drive, go to “Settings” → “Wireless” and choose “Reconnect”.

Signal strength is low (< 10%)

Possible causes:	Solutions:
Distance between the Drive and Wireless Hub is too big.	We recommend keeping both devices in the same room.
Wireless Hub Antennas are loose.	Tighten the antennas.
2.4 GHz band is too crowded.	Try moving other devices (Wi-Fi, Bluetooth) that also use the 2.4GHz band to other areas. Lower their TX-power. Switch Wi-Fi to 5Ghz band. Use a Wi-Fi analyzer app on your smartphone to search for free or less used spectrum.

Experiment cannot be prepared or started in the DOTS Software

Possible causes:	Solutions:
LIS Drive is blocked.	Check which other Object is blocking the Drive.
Experiment details not complete.	Add mandatory information to the experiment details.
No LIS Drive assigned.	Assign a LIS Drive to the experiment.
Assigned LIS Drive is not available.	Make sure the assigned LIS Drive is turned on and properly connected to the LIS Wireless Hub. Make sure that the Device is not blocked by another Experiment (Check the Device list).
LIS Wireless Hub not properly connected.	Connect the LIS Wireless Hub to the DOTS Software.

Why does my Drive switch off or show an Error status?

Possible causes:	Solutions:
Drive pumps too much (battery depletes quickly).	Update parameter set Auto offset the pressure Check for and remove any air bubbles in cartridge outlet Enter correct filling volume in the DOTS Software Check and tighten the Drive/Cartridge assembly
Pressure exceeds safety limit: Filter is wet or cartridge blocked.	Replace the sterile filter. Make sure not to tip the cartridge too drastically during set up.
Battery empty: was not charged, runtime was too long, Drive pumps too much.	See above. Make sure to start a new experiment with a fully charged LIS Drive. Check battery status if running a > 1 weeklong experiment
Computer switched off during run.	Keep computer connected to power during experiment
Wireless Hub not available anymore.	Check PanIds, Wireless connection status, and Hub connectivity with the DOTS Software

LED blink patterns and meanings

LIS Drive

Blink pattern:	Status of Drive:
Orange on 3 seconds during startup	Normal startup behavior (LED test)
Green LED blinking with 2 Hz	Blinking mode triggered by software
Green LED blinking with 1 Hz	Sample mode active
Red LED blinking with 1 Hz	Connecting to Wireless Hub (only when wireless is enabled)
Red LED blinking with 0.1 Hz	Wireless Hub not found (only when wireless is enabled)

Wireless Hub

Blink pattern:	Status of Drive:
Orange on 3 seconds during startup	Normal startup behavior (LED test)
Green LED blinking with 2 Hz	Starting up
Green LED on	Normal operation, no traffic
Green LED blinking	Normal operation, data sent or received
Red LED blinking with 2 Hz	Error: PanID conflict, another device is using the same PanID. Error: Wireless disabled.
Red LED blinking with 10 Hz	Error: internal self-test failed
Red LED lights up during operation	Short pulse: communication with Drive failed Long pulse: communication with computer failed

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Notes

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