tests you can trust

LoopiX®

Instruction manual

Version 3.2 – July 2024

Loop Dee Science SAS
100 rue Philippe LIVRY-LEVEL
14760 Bretteville Sur Odon (France)

CE IVD

Contents

Definitions

Complex signature: Identifiable signature according to several parameters in order to improve its specificity.

Control interface: LoopiX Cloud software or LoopiX App, a computer or smartphone solution for controlling the LoopiX® device.

Deep learning: Type of machine learning based on artificial neural networks.

DNA (Deoxyribonucleic acid): Long molecule composed of a succession of nucleotides, and which contains the genetic information of an individual, of a bacterial species ...

FOM (*Figure of merit*): Mathematical function allowing the measurement of a distance from a typical result.

LAMP: (*Loop-mediated isothermal amplification*): Analytical technique of *in-vitro* gene amplification, close to PCR, which aim is to amplify a specific genetic sequence at a constant temperature.

LoopiX®: Automated molecular biology field analysis device for isothermal amplification (LAMP) and interpretation of results. LoopiX® is used in combination with kits from the LoopDeetect range.

PCR (*Polymerase chain reaction*): An *in-vitro* genetic amplification analytical technique, designed to duplicate in large numbers part of the genetic sequence of a target.

Recipe: Defined by Loop Dee Science according to the target to be detected and the type of sample, the recipe is materialised by software stored in the LoopiX®. The recipe defines the sequence of operations to be performed and the parameters to be applied to distinguish the absence or presence of the target in the reaction tube.

RNA (ribonucleic acid): Molecule with an architecture very similar to DNA. RNAs are synthesized by a living organism on the basis of its DNA and allow a specific function to be performed. In the case of some viruses, RNA directly supports the genetic material (RNA viruses).

RT-LAMP: (*Reverse transcription* LAMP): LAMP analysis suitable for RNA detection.

Sink device: Device capable of receiving data from a source device, in this case, the source device is a computer.

Spectral signature: Electromagnetic emission characteristic of an object as a function of its wavelength.

Thermal lysis: Extraction method which involves heating a sample at high temperature to degrade the membranes of the target to be analysed in order to release its genetic material.

Preamble

The technology implemented via the LoopiX® device is protected by the French patent application: 2003193. Purchase and supply of the device includes neither purchase of a licence to exploit this patent, nor of the know-how belonging to the company Loop Dee Science.

Certain polymerase chain reaction methods imply patented technology. The use of LoopiX® to perform RT-LAMP (apart from detection kits supplied by Loop Dee Science and benefiting from the required rights), may require obtaining intellectual property rights from third parties. It is the sole responsibility of the user to obtain all the necessary rights then.

Technical support

Loop Dee Science provides customer support for any technical questions pertaining to the device. To obtain assistance, please contact our support and assistance team:

Address: Technical support / Customer service at:
Loop Dee Science
100 rue Philippe LIVRY-LEVEL 14760 Bretteville Sur Odon

Telephone: +33 231 730 791 (9.00 am - 5.00pm / Paris time)

E-mail: support@loopdeescience.com

Safety-related information

Before using the LoopiX®, it is important that you read the present instructions for use in order to familiarise yourself with the device. Follow all the instructions to ensure the device's correct operation. Use no other consumable, accessory nor external equipment than those specified. Safety warnings must be adhered to at all times to avoid any risk of bodily injury and/or damage to the device. Should the device be used in a manner not specified by the manufacturer, the protection provided by the equipment may be altered. The advice provided in the present manual is designed to complete, and not to replace, normal safety requirements set forth in the user's country.

Biological safety

Handle all biological material with care and in accordance with required safety regulations. The user must take all necessary precautions to ensure that the surrounding workspace is safe and that device operators are appropriately trained and are not exposed to dangerous levels of infectious agents[1].

Device cleaning

The surfaces of the LoopiX® device, including the cap, must be regularly cleaned by means of an alcohol-based solution or everyday quaternary ammonium-based laboratory disinfectant.

Device decontamination

The surfaces of the LoopiX® device, including the chamber and the cap, can be decontaminated by means of a sodium hypochlorite solution (NaOCI). A solution containing 1 g/L of available chlorine will suffice; stronger solutions (5 g/L) are recommended in high-risk situations[2].

Waste disposal

Waste disposal must conform to all national, regional and local laws and regulations pertaining to health and safety.

Applicable standards and directives

- The LoopiX® device uses a 5V/3A low-voltage power supply and requires no particular measures with regard to the Low Voltage directive 2014/35/EU.
- The LoopiX® is compatible (Sink device) with the EN IEC 62680-1-2 (USB power delivery) standard.
- A power supply (wall-plugged charger) is provided with the device and is compatible with the aforementioned standard and the Low Voltage directive 2014/35/EU.

[1] Biosafety in Microbiological and Biomedical Laboratories,(http://www.loopdeescience.com/download/26/notices/833/bmbl_v6-0.pdf)

[2] World Health Organization. Laboratory Biosafety Manual – 3rd ed. Geneva: World Health Organization; 2004.

General characteristics of the LoopiX® device / Application scope

The LoopiX® is a compact automatic amplification, reading and interpretation of results, allowing to perform a LAMP or RT-LAMP.

It enables the amplification of a biological target DNA or RNA sequence (virus, bacteria, etc.), introduced into a reaction tube containing reagents that are specific to the target, in order to obtain sufficient copies to detect an exploitable signal to confirm the presence (positive) or absence (negative) of this target.

The reaction tube is heated by means of a Joule heating system and cooled by forced airflow. Thanks to our patented technology, the LoopiX® is fast enough to complete a full reaction, followed by detection, within 30-50 minutes.

The detection of the target DNA or RNA sequence at the end of the reaction is measured by fluorescence. The device is equipped with a 480 nm excitation channel and a multichannel sensor for wavelengths from 415 to 680 nm.

Reaction parameters (temperature and duration), fluorescence signal measurement and identification of the spectral signature and its intensity, are grouped together in a 'recipe' established by Loop Dee Science and programmed within the device.

The LoopiX® device has been designed to limit any handling risks as well as to render operation simple. It gives a result in a totally autonomous manner and can operate without being connected to a computer.

The LoopiX app and LoopiX Cloud online software can be used to manage the recipes developed, enabling the entire range of LoopDeetect products to be used.

It is designed for exclusive use with LoopDeetect reaction tubes and kits.

Detailed description

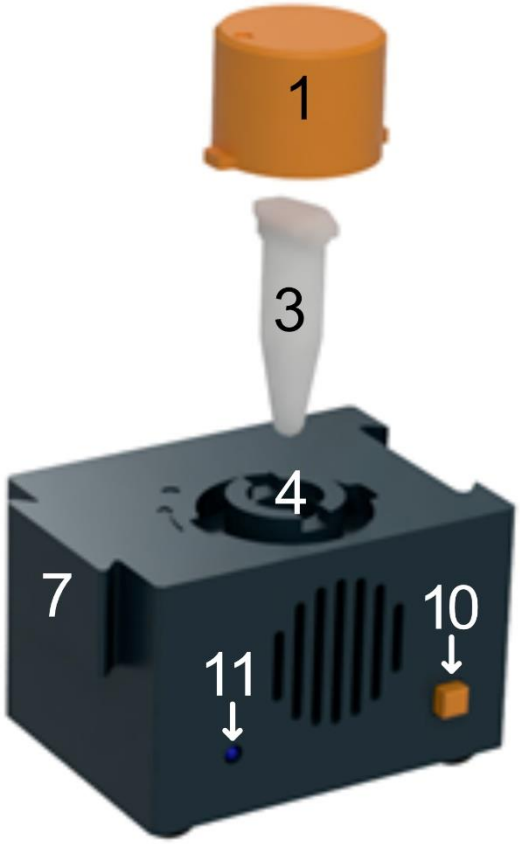

Figure 1a: Front view

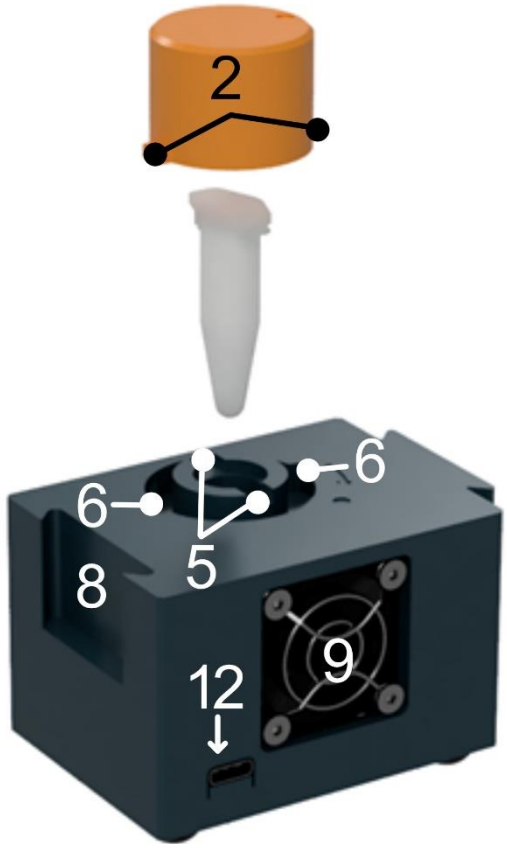

Figure 1b: Rear view

1	Cap	**7**	Tenon to assemble several LoopiX® devices together
2	Cap positioning notches	**8**	mortise to assemble several LoopiX® devices together
3	Reaction tube (this tube is provided in each LoopDeetect kit)	**9**	Fan
4	Reaction well	**10**	Control button
5	Reaction tube positioning guides	**11**	Indicator light
6	Cap insertion notches	**12**	USB-C Connector

Figure 1 : LoopiX® description

Cap (1): equipped with positioning notches (2), is used to ensure that the reaction well (4) is closed:

- Before starting analysis, the operator must take care to correctly close the cap (1) and hence sealing the reaction well (4), to prevent stray light from distorting the result.
- This cap (1) fits into the insertion notches (6), visual and sound cues ('click') will ensure its correct positioning.
- The user is informed of any error (flashing red light after pressing the control button (10)), should the cap (1) be inadvertently incorrectly positioned.

Reaction tube (3): the reaction tube (3) must be inserted into the reaction well (4), following the reaction tube positioning guides (5).

Reaction well (4): this well houses the reaction tube (3). The reaction well (4) is then closed by means of the cap (1).

Fan (9): placed at the back of the device and to the right of the USB-C connector. It ensures cooling of the reaction well (4).

Control button (10): located on the front face of the device, the control button (10) enables, via simple pressure, to:

- Launch an analysis,
- Stop it in the case of a handling error observed by the operator,
- Relaunch a new test after reading the result of the previous one.

Indicator light (11): The indicator light is designed to inform the user of the ongoing phase and to guide the user on the device's use. The following table summarises the various colours that may be displayed.

Colour	Status	Interpretation
White	Steady	At start-up: LoopiX® performs a self-check on the status of its functions.
Blue	Steady	The device is ready for use
Blue	Flashing	At start-up: LoopiX® is heating/cooling to reach its set temperature. Analysis: LoopiX® is waiting for an action from the user.
Yellow	Flashing	Analysis is in progress
Red	Flashing	LoopiX® performs extraction by thermal lysis
Green	Steady	Analysis is complete, the sample is negative, no target presence detected (virus, etc.)
Red	Steady	Analysis is complete, the sample is positive, target presence detected (virus, etc.)
Red and green	Flashing	The analysis is complete, the result is uninterpretable, linked to sample inhibition or improper handling in the pre-amplification steps **(functionality only available with kits with internal control).**
Purple	flashing	At start-up: the self-check revealed an anomaly At the start of an analysis: problem downloading the recipe, disconnect and reconnect LoopiX, then start again. During the process: The time limit between the extraction phase and analysis has been exceeded.
Purple	Steady	Device failure (contact our customer support)

Table 1 : Meaning of indicator light colours

Power supply / USB-C Connector (12): houses a USB-C cable (refer to Figure 2) in conformity with the EN IEC 62680-1-2 (USB power delivery) standard, enabling the LoopiX® device to be powered. This power connection can be supplied by means of any wall socket, computer or portable battery compatible with the aforementioned standard:

- The necessary power is 15W (5V x 3A).
- A compatible power supply, in conformity with the standard, of a maximum power of 18W, is provided with the detection device.
- Independent batteries are available, enabling the device to operate in the absence of a land-based power supply.

Figure 2 : USB-C standard cable connector

Operating principle and comments

1) Prepare the analysis according to the procedure of the LoopDeetect kit used.
2) The tube (3) is placed in the well (4).
3) LoopiX® analysis is started by pressing the control button (10) or by pilot using the control interface (see chapter Detailed operating procedure).
4) For an extraction by thermal lysis, subsequent operations follow by means of a microcontroller included in the device.
    a. LoopiX® heats up to the temperature defined in the recipe.
    b. The tube is kept at this temperature throughout the time defined by the 'recipe'.
    c. LoopiX® cools to 30°C
    d. The light changes state (flashing blue) to indicate thermal lysis is complete and the sample is ready to proceed to the next stage. The operator can then continue with the manipulations.

NB: The waiting time is limited to avoid sample degradation. If the analysis has not been completed within the allotted time, the light will turn flashing purple and the analysis will have to be restarted from the beginning with a new sample.

5) For an amplification, subsequent operations follow by means of a microcontroller included in the device.
    a. A series of measurements is conducted in order to:
        i. Check the presence of the cap (1).
        ii. Calibrate detection.
    b. The reaction tube is heated to the temperature defined in the 'recipe'.
    c. The reaction tube is kept at this temperature throughout the time defined by the 'recipe'.
    d. The reaction tube is then cooled.
    e. A series of measurements is conducted to detect the fluorescence signal.
    f. After calibrating the measurement, spectral signals are compared with the expected signature of a positive reaction and to produce two parameters:
        i. A list of coefficients relating to the fluorescences we are looking for. These values are proportional to the intensity of the targeted signatures..
        ii. A threshold value Kx which characterises the mathematical distance between this expected signature and the measured signature. A "Positive" result will always have a Kx greater than or equal to the defined Kx. A "Negative" result will have a Kx strictly less than the defined Kx.
        iii. In the case of multiplex analyses, there are as many Kx as there are signatures being looked for :

1. K1 for a Simplex
2. K1 and K2 for a duplex
3. K1 to K_X for a multiplex

6) The result is reported to the user by means of the indicator light (11):

Indicator light	State	Verdict	Target	Reaction control (only with a kit with internal control)
Red	Steady	Positive	Detected	Detected
Red	Steady	Positive	Detected	Not Detected*
Green	Steady	Negative	Not detected	Detected
Red/Green (only with kits with internal control).	Flashing	Uninterpretable	Not detected	Not detected

*In rare cases, the target may be detected but not the internal control. In this case, the diode will also be solid red

Table 2: Interpretation of the indicator light

The Gradient Descent algorithm (JONATHAN BARZILAI, JONATHAN M. BORWEIN, Two-Point Step Size Gradient Methods, *IMA Journal of Numerical Analysis*, Volume 8, Issue 1, January 1988, Pages 141–148, https://doi.org/10.1093/imanum/8.1.141) used to determine the FOM and coefficients is standard, abundantly documented in the literature and widely used in 'Deep Learning' and neuronal network contexts.

The technology embedded in the LoopiX® device offers several advantages:

- One particular advantage, over and above the speed of convergence (a few milliseconds in the case of LoopiX®), is that it provides two parameters, FOM and coefficient, to interpret a result and to distinguish the presence or absence of the target.
- The multispectral technology (8 bands) used in the LoopiX® also provides a complex signature, to avoid it being confused with any other signature indicating the presence of the target.

Caution

The LoopiX® and kits (reagents and operating procedure) have been designed so that the reaction tube never requires to be opened once the LAMP amplification step is complete.

In order to avoid contamination of the test environment by target DNA or RNA, which can impact subsequent tests, we recommend that you never open the reaction tube without applying necessary and adequate protective measures (high risk of false positive results).

Specifications

Physical	Dimension	72 x 53 x 68mm (sealing cap included).
	Weight	141g
Electrical	DC	5V / 3A
Thermal performance	Temperature stability	± 0.25°C (+/- 0.45°F)
	Temperature range	30°C – 70°C 86°F - 158°F
	Temperature ramp	1°C - 1.8°F/s (heating)
		1°C - 1.8°F/s (cooling)
Optical	Detection	Multispectral sensor
	Excitation	LED 480 nm
Conditions for use	Temperature	10°C – 25°C (50°F - 77°F)
	Humidity	20 – 80% without condensation
Storage conditions	Temperature	- 20°C to + 80°C (-4°F to +176°F)
	Humidity	20 – 80% without condensation

Table 3 : LoopiX® technique specifications

Detailed operating procedure

1. **The operator must always ensure that temperature conditions are guaranteed: 10°C - 25°C (50°F - 77°F)**
2. Connect the LoopiX® to a USB Power Delivery-compatible power source (5V/3A current) using the USB-C connector (12) and a USB-C cable. A power supply for a wall plug is provided with the device.
3. LoopiX® switches on, the fan (9) starts up briefly and the status LED (11) lights up steadily in white, indicating the self-test phase.
4. If the auto-check is successful, the status indicator (11) first flashes blue and then lights up steady blue.
5. Using the control interface, load the recipe corresponding to the LoopDeetect kit being used. In some kits in the LoopDeetect range, depending on the operating mode, the control interface will ask what type of analysis is to be performed:
    - **Lysis then amplification**: The loopix will perform thermal lysis, then a user action will be required to continue with the amplification step. Continue the protocol from step 6.
    - **Amplification** : LoopiX® will perform the amplification directly. Continue the protocol from step 11.

    NB: By default, the LoopiX® executes the last loaded recipe.
6. Prepare the sample by scrupulously following the procedure of the LoopDeetect kit used.
7. Place the compatible (LoopDeetect) tube (3) in the LoopiX® well (4), taking care to position it in the indicated imprint.
8. Position the cap (1) by:
    - Lining up the two cap positioning notches (2) with the cap insertion notches (6),
    - Vertically pressing the cap (1) to the limit,
    - Turning the cap (1) clockwise up to the positioning marks. The cap will make an audible clicking noise when correctly positioned.
9. Start the lysis step by briefly pressing the control button (10) or via the control interface. The status LED (11) lights up red and flashes throughout the process.
10. At the end of lysis, LoopiX® cools down and waits for an intervention before moving on to amplification. LoopiX® flashes blue for a limited time defined in the recipe.
11. Proceed according to the procedure for the LoopDeetect kit used, place the reaction tube in the LoopiX®.
12. Start the analysis by briefly pressing the control button (10). The indicator light (11) flashes yellow throughout the entire analysis process.
13. At the end of the amplification cycle, the well (4) is cooled by activation of the fan (9), which stops amplification and enables the fluorescent measurement to be taken.

14. When the fan (9) stops at the end of the cooling phase, the LoopiX® conducts the fluorescence measurement phase.
15. Then proceed to analysis of the spectral signature in order to calculate the required parameters for determining the detection or non-detection of the target.
16. The result can then be read directly by the statut of the indicator light (11):

Indicator light	State	Verdict	Target	Reaction control (only with a kit with internal control)
Red	Steady	Positive	Detected	Detected
Red	Steady	Positive	Detected	Not Detected*
Green	Steady	Negative	Not detected	Detected
Red/Green (only with kits with internal control).	Flashing	Uninterpretable	Not detected	Not detected

*In rare cases, the target may be detected but not the internal control. In this case, the diode will also be solid red

Table 2 (recall): Interpretation of the indicator light

17. Record the result
18. Remove the cap (1) by:
    - Pressing the cap (1) to the limit.
    - Turning it anti-clockwise until the cap positioning notches (2) are lined up with the cap insertion notches (6).
19. Remove the reaction tube (3) and dispose of it as per your internal procedures.

    <u>Never open the tube.</u>
20. Press the control button (10) again.
21. The LoopiX® is ready for a new measurement (blue light (11) and return to step 3).

Recommendations and warnings

- In order to guarantee optimal operation, the LoopiX® must be used at room temperature (10°C to 25°C (50°F to 77°F)) and away from direct sunlight.
- Do not use LoopiX® to perform tests with kits that are not supplied by Loop Dee Science.
- Do not run an analysis with an empty LoopiX® or an empty tube, as this may damage the device.
- LoopiX® should be placed horizontally on a flat surface.
- Detection kits and the LoopiX® device must be used only as indicated in the instructions. Loop Dee Science World Wide accepts no responsibility for any consequences arising from improper use or failure to comply with the manufacturer's recommendations concerning the LoopiX® device.

- During analysis, do not unplug, open, disconnect or interrupt LoopiX® until the analysis is complete.
- LoopDeetect kits may only be used once and must be disposed of after use.
- Never open a reaction tube. This presents a risk of disseminating target genetic material, hence yielding exclusively positive results on subsequent tests. Such precaution is a matter of good laboratory practise for the biologist and laboratory technician. It should be noted that the design of the LoopiX® detector and kits does not require for the reaction tube to be opened to analyse results. This considerably limits the likelihood of this frequently encountered risk.
- Several LoopiX® detectors may be advantageously 'connected' by means of tenons and mortises (7 and 8), hence enabling testing speed to be accelerated.
- Should no mural USB-C source be available, the LoopiX® can be correctly and sufficiently powered by means of a portable battery.

Troubleshooting

Observation	Possible causes
The device does not start, the status LED does not show any light.	- Faulty power supply - Use of a cable and/or plug not compatible with the appliance.
When the device is plugged in, the ignition cycle repeats itself indefinitely	LoopiX is plugged directly into the computer's USB port. The power source is not compatible with LoopiX
The fan runs continuously / When switched on, LoopiX remains flashing blue	LoopiX cannot reach its set temperature (30°C) because the room temperature is too high.
The fan does not start during cooling phases	Device out of order, contact your dealer.
Status light is flashing purple	On switching on: The autocheck has revealed an anomaly. Check that the plug on the controller is intact and correctly positioned and that the ambient temperature in the room does not exceed 25°C. Reconnect the device and if the problem persists, contact your dealer. At the start of an analysis: the recipe was downloaded incorrectly, disconnect and reconnect the device, then repeat the operations. Between extraction and amplification: the waiting time has been exceeded, the sample is no longer viable. At the verdict (after amplification): The loopiX is unable to reach its set temperature (30°C) and has not been able to perform the reading.

Document history / Versions

Revision	When	Comments
1.0	1 September 2020	Initial 'public' revision
1.1	2 October 2020	Correction of the operating temperature range. 10°C-25°C (50°F-77°F) instead of 15°C-30°C (59°F-86°F). Addition of a recommendation for operating temperature at the start of the 'Detailed operating procedure'.
1.2	12 March 2021	Addition of the Definitions chapter Replacement of Figure # 1 and Figure # 2. Replacement of the link in the chapter "Instrument decontamination". Spelling corrections and semantic modifications.
1.3	08/06/21	Spelling corrections. Minor semantic changes.
2.0	8 August 2021	Modification of the algorithm for calculating spectral signals in the chapter "operating principle and commentary" Modification of tables 2; 3; 5 for the addition of the alternating red / green flashing Adding soft LoopiX® orthographic corrections and semantic modifications.
2.1	16 September 2021	Address modification
3.0	10 February 2023	Change of name from LoopDeeLab to LoopiX®. Addition of extraction specifications Modification of the "Meaning of LoopiX® status indicators" table Addition of the LoopiX App application Modification of the "Operating principle and comments" section Advice and warnings" section updated Removal of the IVD symbol - development of non-CE IVD PLC applications
3.1	May-24	Modification of links LoopiX cloud added Change in flashing purple status
3.2	July-24	Troubleshooting chapter added

Table 4: Versions history