BioVendor Group LAMP

ENG

Product Data Sheet:

# SARS-CoV-2 Direct LAMP 96-KIT

Catalogue number:

**RDLAMP0001**

European Union:

CE IVD

Rest of the world:

**For research use only!**

**For professional use.**



BioVendor R&D®

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# CONTENTS

# 1. INTENDED USE

RDLAMP0001 BioVendor SARS-CoV-2 Direct LAMP 96-kit is intended for the detection of SARS-CoV-2 viral nucleic acid causing Covid-19 disease and is based on the principle of the RT-LAMP method

## Features

- **European Union: For in vitro diagnostic!**
- **Rest of the world: For research use only!**
- The total assay time is less than **45 min**
- The use of SARS-CoV-2 Direct LAMP 96-kit allows the **isolation free detection** of viral nucleic acid and allows the sample analysis directly after a short thermal inactivation
- The method is intended for screening of persons for the presence of SARS-CoV-2 virus
- The kit detects SARS-CoV-2 virus nucleic acid from nasopharyngeal swabs stabilized with **non-inactivating** Viral Transport Media (VTM)
- The kit capacity is 96 reactions

## Abbreviations

| | |
|---|---|
| Ct | Cycle threshold |
| FAM | 6-carboxyfluorescein |
| LoD | Limit of Detection |
| min | minute |
| N | Not detected |
| N/A | Not applicable |
| NC | Negative Control |
| NFW | Nuclease Free Water |
| PC | Positive Control |
| RFU | Relative Fluorescent Unit |

## 2. STORAGE, EXPIRATION

Store the kit at -20 °C. Under these conditions, all components are stable until the expiration date (see label on the box).

- The SARS-CoV-2 Direct LAMP 96-kit is delivered frozen at -20 °C.
- After delivery store the SARS-CoV-2 Direct LAMP 96-kit at -20 °C.
- **Protect kit components from light**.
- Reagents may be aliquoted if necessary.
- Avoid repeated freeze-thaw cycles.
- Do not use the kit after the expiration date.
- For stability of opened reagents see Chapter 9.

## 3. INTRODUCTION

SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) is an enveloped RNA virus of the *Coronavirideae* family. It is the causative agent of Covid-19, which spread worldwide from China's Hubei province at the end of 2019. In January 2020, a global health threat was declared by the World Health Organization (WHO) [1].

The virus is spread by contact with each other, primarily through droplets, which the infected person excretes when coughing, sneezing or speaking. Infection can occur by inhaling or transmitting the virus in a droplet from the surface to the face (typically by touching the mouth, nose or eyes). It is not clearly determined how long the virus survives outside the human body [2].

The incubation period is between 1 and 14 days, the usual time from infection to the first symptoms is 5 to 6 days. The main symptoms include dry cough, shortness of breath, fatigue and fever, more severe cases can lead to pneumonia, kidney failure and even death. Symptoms usually come gradually and about 80 % of patients recover without the need for hospitalization. The disease progression and severity also depends on the overall health and physical condition of the patient [3].

The virus naturally mutates during spread in the population and variants with higher transmission ability become dominant [4]. One of the fastest spreading mutations was identified as a variant called B.1.1.7 (SARS-CoV-2 VUI 202012/01 according to the WHO classification, also referred as 501Y.V1), known as the British mutation [5].

Reliable and rapid testing of the population for the presence of the virus is a critical step in stopping the spread of the disease and stabilizing the pandemic situation.

## 4. TEST PRINCIPLE

The SARS-CoV-2 Direct LAMP 96-kit is an *in vitro* test kit for the detection of SARS-CoV-2 RNA based on the principle of amplification of viral RNA under isothermal conditions. The reaction takes place in a single tube at constant temperature and uses the principle of the Reverse Transcription Loop Mediated Isothermal Amplification (RT-LAMP). The input material for the LAMP reaction can be directly the sample medium from the collection tube without the need of viral RNA isolation. In the LAMP reaction, the target sequence is amplified at constant temperature using a set of primers and a fluorescently labelled displaceable probe, in the presence of a polymerase which, in addition to replication activity, also has the strand dissociation ability. The presence of loop primers increases the rate and dynamics of the reaction, so the amount of nucleic acid generated in LAMP reactions tends to be higher than in classical thermocyclic amplification, and detection of the target sequence may be more than half faster. Detection of the presence of target sequence is mediated by the fluorescence elevation produced by isothermal amplification on an optional platform detecting the FAM channel (480-530 nm).

## 5. PRECAUTIONS

- **For professional use!**
- The kit components do not contain infectious material.
- All samples for testing with the SARS-CoV-2 Direct LAMP 96-kit should be handled as if they are infectious, following conventional biosafety precautions.
- Use respiratory, hand and eye protection when working (respirator FFP2 and higher, protective disposable gloves, goggles or shield).
- Do not drink, eat or smoke in areas where biological material is handled.

## 6. TECHNICAL HINTS

- The kit is validated on samples of nasopharyngeal swabs collected into **non-inactivating** VTM medium. The use of samples obtained by other sampling procedures requires in-house validation.
- The sample for analysis with the SARS-CoV-2 Direct LAMP 96-kit **must NOT be inactivated in any way** (chemically or thermally). Inactivation is a part of the test workflow. Any deviations may result in analytical properties failure or cause erroneous results of the test.
- The kit is intended for use by professional users in an adequate laboratory environment.
- Before and after each test, the working environment must be decontaminated with standard RNase, DNase disinfectant. Working in an unsuitable environment can lead to contamination of the kit components.
- Each kit is designed for 96 reactions. It is always necessary count the reserve for performing positive and negative controls. A maximum of 94 samples + 2 controls can be measured by one kit.

  **The number of required reactions is n + 2 (n = number of samples).**
- LAMP Master Mix is supplied in two aliquots, each is sufficient for 48 reactions. We do not recommend repeat more than 2 freeze-thaw cycles, multiple thawing may affect test quality and results.
- Let individual kit components thaw just before use. Minimize the time that the reagents are at room temperature. We recommend the use of cooling racks.
- Avoid repeated freeze-thaw cycles of reagents.
- Vortex gently the reagents before using.
- **Never open the Positive Control tube during test preparation**, **pipette it always as the last part of the analysis**. Improper handling may result in contamination of the test and false positive results. If contamination is suspected, repeat the test.
- Add a positive (PC) and negative control (NC) to each run of the SARS-CoV-2 Direct LAMP 96-kit to evaluate proper reaction preparation and exclude contamination. In case of non-compliance, false positive or negative results cannot be ruled out.
- SARS-CoV-2 Direct LAMP 96-kit targets the **S region** of the viral RNA, using another synthetic molecule that does not contain the region as a positive control, may lead to a false negative result.
- Avoid cross contamination of samples and reagents. Use disposable pipette tips for each sample and reagent.

- Do not open the tubes after the LAMP reaction and dispose used material in accordance with the legislation.
- Do not mix reagents with different LOT numbers.

## 7. REAGENT SUPPLIED

The kit is supplied as "ready to use" to perform 96 reactions (Table 1). The kit includes a **LAMP Master Mix** containing all the necessary components of the reaction, then **Positive Control (PC)**, which is used to check the correct performance of the analysis and **Nuclease Free Water (NC)** as a negative control of the reaction (exclusion of contamination).

| Component | 1 tube volume (µl) | Number of Tubes | State |
|---|---|---|---|
| **LAMP Master Mix** | 1320 | 2 | ready to use |
| **Positive Control** | 50 | 1 | ready to use |
| **Nuclease Free Water** | 100 | 1 | ready to use |

*Table 1: Components of the SARS-CoV-2 Direct LAMP 96-kit*

## 8. RECOMMENDED MATERIAL (NOT SUPPLIED)

- Commercially available solutions for surface decontamination
- Disposable gloves
- **For nasopharyngeal swab samples:** Disposable Collection Tubes of Virus Samples (Jiangsu Mole Bioscience)
- 1,5 - 2 ml tubes suitable for work with nucleic acids (RNAse + DNase free, low binding nucleic acid tubes)
- PCR tubes / strips / plates suitable for fluorescence detection instrument (suitable size, profile and light transmittance) – transparent, colourless plastics marked "suitable for PCR" are recommended
- Thermocycler for measuring fluorescence with the FAM channel (480 - 530 nm) with heating to 65 °C
- Cooling rack / fridge / freezer / ice box for cooling tubes after inactivation
- Automatic pipettes for volumes 5 - 100 µl, or automatic dosing pipes for volumes 5 - 25 µl
- Disposable filter tips
- Vortex, centrifuge for tubes with a volume of ≤ 2 ml
- Thermoblock / incubator / water bath with heating to 95 ° C
- Test tube racks

## 9. PREPARATION OF REAGENTS

– **Always prepare only the appropriate quantity of reagents for your test.**

– **Do not use components after the expiration date marked on the label.**

– **Reagents are supplied as ready to use.**

### LAMP Master Mix

Let thaw enough LAMP Master Mix for the batch of samples just before preparing the reaction and keep it cool prior to use.

Stability and Storage: LAMP Master Mix is supplied in two aliquots; each is sufficient for 48 reactions. Always store at -20 °C and protect from light. It is not recommended to repeat more than two freeze-thaw cycles of LAMP Master Mix aliquots, multiple thawing may affect test quality and results. Freeze the reagent immediately after using the aliquot. Opened LAMP Master Mix is stable 6 months stored at -20 °C.

### Positive Control

Let the Positive Control thaw at room temperature before preparing the reaction. Mix and briefly centrifuge before use to prevent liquid from sticking in the lid.

***CRITICAL NOTE:*** ***Never open the Positive Control tube during test preparation****, always* ***pipette it as the last part of the reaction. Improper handling may result in contamination of the test and false positive results. If contamination is suspected, repeat the test.***

Stability and Storage: Positive Control is supplied in a volume of 50 ul, for multiple use it is possible to prepare aliquots. Always store at -20 °C. It is not recommended to repeat more than four freeze-thaw cycles of Positive Control aliquots, multiple thawing may affect test quality and results. Opened Positive Control is stable 6 months stored at -20 °C.

**Nuclease Free Water – negative control**

Let thaw the tube with negative control (Nuclease Free Water) at room temperature before preparing the reaction. Mix and briefly centrifuge before use to prevent liquid from sticking in the lid.

Stability and Storage: Nuclease free water is supplied in a volume of 100 ul, for multiple use it is possible to prepare aliquots. Always store at -20 °C. Opened Nuclease free water is stable 24 months stored at -20 °C.

## 10. PREPARATION OF SAMPLES

The kit detects SARS-CoV-2 viral nucleic acid from nasopharyngeal swabs collected to **non-inactivating** VTM (recommended composition according to CDC SOP#: DSR-052-05, available at https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf). The use of another form of input sample requires in-house validation.

Samples should be analysed within 24 hours after collection, long-term storage of samples may affect test quality and results.

**Respect the collection, handling and storage recommendations according to the collection kit manufacturer.**

Generally, the use of nasal spray is not recommended before collection of nasopharyngeal swab (follow the instructions of the collection kit manufacturer).

The sample for analysis with the SARS-CoV-2 Direct LAMP 96-kit **must NOT be inactivated in any way** (chemically or thermally), or degraded (e.g., by dilution, or by the addition of other buffers, etc.). Inactivation is a part of the test workflow.

1. Mix the sample in a collection tube and centrifuge briefly (make sure the sample does not stick in the lid).

2. Pipette **50 µl of sample into an empty tube (1.5 - 2 ml).** If the sample is not homogeneous or contains solid particles, centrifuge repeatedly.

3. **Inactivate** the sample with heat step **in the tube at 95 °C for 5 minutes.**

4. **Cool** the sample briefly **to 4 °C.**

5. Spin down the tube briefly.

6. The sample is **ready for analysis.** Proceed according to chapter 11. Assay Procedure.

## 11. ASSAY PROCEDURE

1. Prepare reaction tubes for appropriate number of samples (n) + 2 extra for PC and NC. **Number of tubes (reactions) = n + 2**

   ***Precaution**: Do not label the tubes, it is necessary to allow fluorescence to be read without interference.*

2. Pipette **25 µl of LAMP Master Mix** into each tube.
3. Pipette **5 µl of each sample** into the appropriate tube.
4. Pipette **5 µl Nuclease Free Water** into an NC tube.
   Pipette **5 µl Positive Control** into a PC tube.

   ***Precaution**: **PC is always pipetted as the last part of the analysis, there is a risk of contamination!***

5. Centrifuge the reaction tubes briefly (make sure the reaction mixture is at the bottom of the tubes).

   *An example of a test layout is available as Appendix 1.*

6. Place the reaction tubes with the prepared reaction mixture in a suitable detection device (fluorescent thermocycler is recommended).

7. Set the reaction program according to Table 2 and start the reaction.



| Type of device | Step | Temperature | Time | Fluores-cence |
|---|---|---|---|---|
| **Real-time thermocycler** (e.g. BioRad CFX 96, MIC, Roche 480 Light Cycler) | 1 | 65 °C | 1 min (+ fluorescence reading) | FAM (480 – 530 nm) |
| | 2 | GO TO 1 | 30x repeat | |

*Table 2: Program for reaction*

## 12. RESULTS EVALUATION

### A) Positive Result

A positive result of LAMP amplification in the sample is any increase in the fluorescent signal above the normal background level during reaction time (e.g. S-shaped curve). An example of positive sample signal from a thermocycler is shown in Figure 1.

The detection of LAMP amplification is **qualitative**, the value of the detection time (min) corresponds to the Ct value read by the thermocycler. This time value may not correspond linearly to the amount of template at the beginning of the reaction and is only informative value of the viral load.

**Interpret the sample as positive when reading detects any increase in fluorescence signal above the background level during the reaction.**

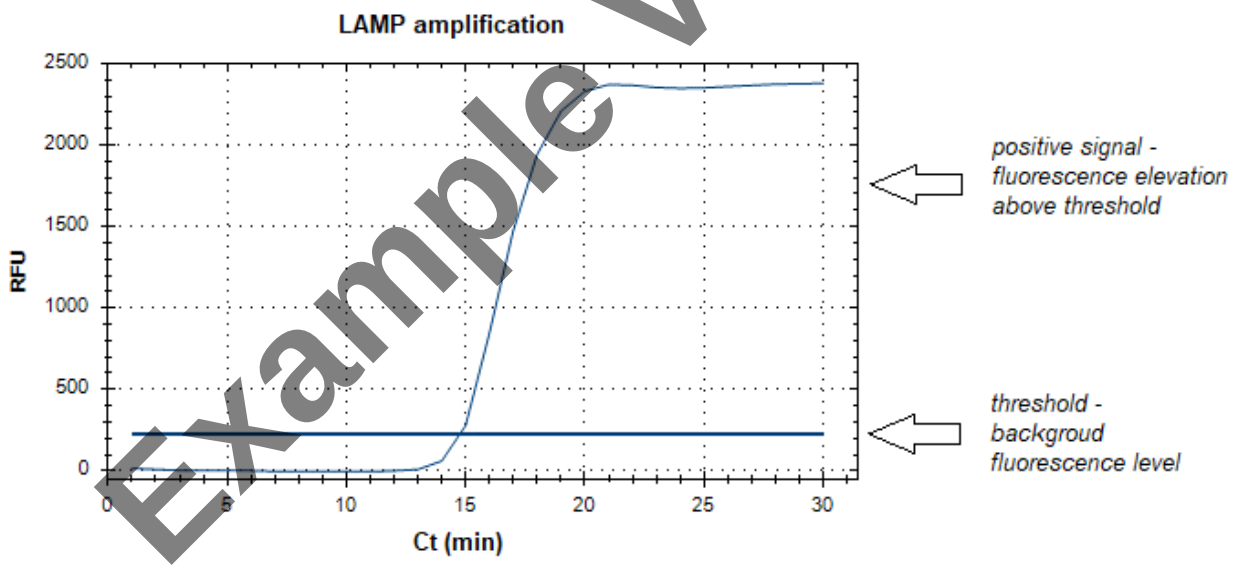


*Figure 1: The fluorescence curve record (positive signal) for the positive sample or the correctly measured PC.*

## B) Negative result

If there is not SARS-CoV-2 virus in the sample at the beginning of the reaction, no change in the fluorescence signal is detected.

**Interpret the sample as negative when there is no detection of the fluorescent signal during the entire reaction time.** The example of the thermocycler record for negative sample is shown in the Figure 2.

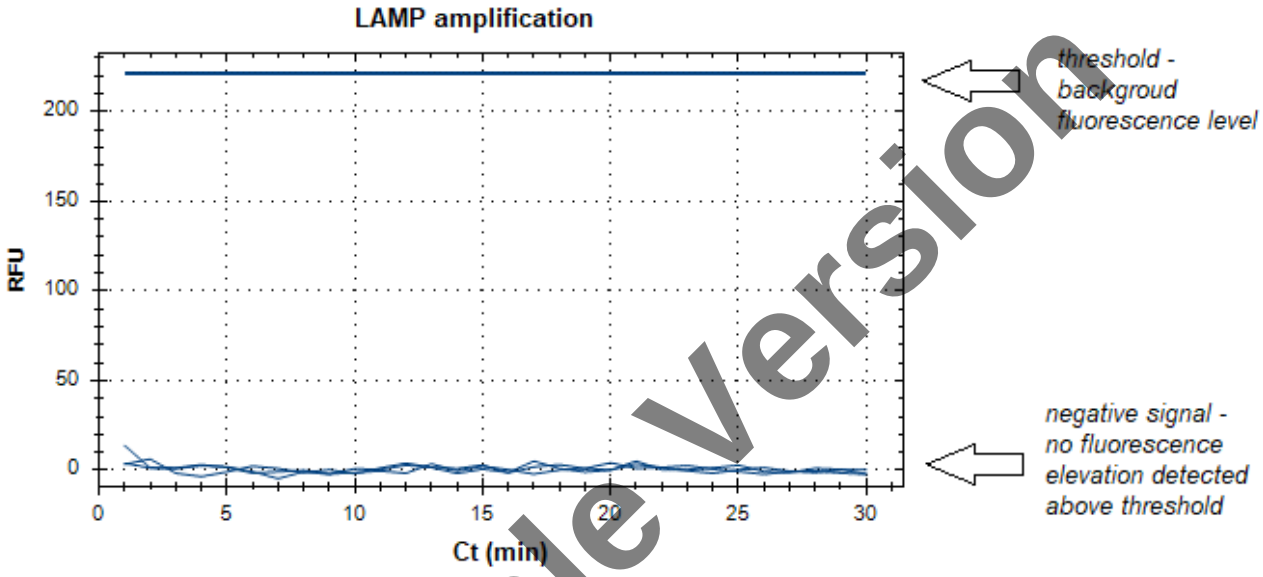


*Figure 2: The fluorescence record without the detection of fluorescence signal (negative signal) for negative sample and the correctly measured negative control.*

## C) PC a NC interpretation

Inclusion of Positive and Negative controls for each test run (group of samples measured simultaneously) **is necessary** to eliminate technical problems during the reaction and to correctly interpret the results (Table 3).

PC is detected as positive signal in the PC tube.

NC is detected as negative signal in the NC tube.

If **no positive signal is detected in the PC tube for the test run, the reaction did not proceed properly**, and the test must be repeated.

If **positive signal is detected for the NC tube in the test run, the reaction was contaminated**, and the results are not reliable. The test run must be repeated.

*More information in chapter. 15: Troubleshooting and frequently asked questions.*

| PC | NC | Result | Solution |
|---|---|---|---|
| positive | negative | test valid | test is valid |
| positive | positive | test invalid | chapter 15, n. 1 |
| negative | positive | test invalid | chapter 15, n. 2 |
| negative | positive | test invalid | chapter 15, n. 3 |

*Table 3: Test results interpretation based on the results of PC and NC*

## 13. TEST LIMITATIONS

- The SARS-CoV-2 direct LAMP 96-kit is validated on nasopharyngeal swab samples collected in VTM medium (Disposable Collection Tubes of Virus Samples, Jiangsu Mole Bioscience).
- The quality of the measurement is affected by the quality of the sample**.** Proper sample collection, transport and storage procedures are critical for the measurement.
- Test results should be evaluated by a healthcare professional with respect to the patient's medical history, clinical condition, and other diagnostic results.
- SARS-CoV-2 Direct LAMP 96-kit is designed specifically for the detection of SARS-CoV-2 virus, a negative result does not exclude the infection by other pathogens.
- SARS-CoV-2 Direct LAMP 96-kit is intended for screening the population for the presence of SARS-CoV-2 virus, a negative result does not exclude a weak infection with the amount of virus below the limit of detection of the method.
- All instructions in this document should be followed when performing measurements. Any deviations may affect the quality and reliability of the results.

# 14. KIT CHARACTERISTICS

## 14.1 ANALYTICAL FUNCTION

This chapter presents the characteristic analytical data of the SARS-CoV-2 Direct LAMP 96-kit manufactured by BioVendor.

### Analytical sensitivity (LoD)

Limit of detection (LoD) of SARS-CoV-2 Direct LAMP 96-kit was tested using the synthetic nucleic acid sequence of SARS-CoV-2 (Twist Synthetic SARS-CoV-2 RNA Control 1, cat. n. MT007544.1 a First WHO International Standard for SARS-CoV-2 RNA NIBSC, cat. n. 20/146) in serial dilution. Based on the results, the LoD of the kit was determined to be **$10^2$ copies/µl**. A positive signal for this concentration was detected in all replicates.

The LoD of the method in the real conditions was tested on a series of positive samples determined by the RT-qPCR method, segregated into groups of strongly positive (Ct 18 – 25), medium positive (Ct 26 – 34) and weakly positive (Ct ≥ 35) samples. By comparing the results of the RT-qPCR method, which were considered as correct for the purposes of this test, the LoD of SARS-CoV-2 Direct LAMP 96-kit corresponded to the PCR determination until the cycle 35 (**Ct < 35)** (Table 4).

| PCR (Ct) | sample | sample type | LAMP result | RT-qPCR (Ct) | PCR result |
|---|---|---|---|---|---|
| Ct ≤ 25 | 1 | nasopharyngeal swab | positive | 23.5 | positive |
| | 2 | | | 18.8 | |
| | 3 | | | 20.7 | |
| | 4 | | | 25.5 | |
| Ct = 26 – 34 | 5 | nasopharyngeal swab | positive | 32.4 | positive |
| | 6 | | | 34.1 | |
| | 7 | | | 27.1 | |
| | 8 | | | 28.7 | |
| Ct ≥ 35 | 9 | nasopharyngeal swab | negative or below LoD | 35.6 | positive |
| | 10 | | | 39.7 | |
| | 11 | | | 35.1 | |
| | 12 | | | 35.1 | |
| | 13 | | | 37.3 | |
| | 14 | | | 42.3 | |
| | 15 | | | 38.7 | |
| | 16 | | | 38.5 | |

*Table 4: Determination of the detection limit of the SARS-CoV-2 Direct LAMP 96-kit on real samples – comparison with RT-qPCR method*

## Analytical specificity

Verification of cross-reactivity was performed *in silico* by comparing the sequences of related and other possible interacting microorganisms with the sequences of primers and probes providing specific detection of SARS-CoV-2 virus by SARS-CoV-2 Direct LAMP 96-kit technology. It was verified that the only possible cross-reaction could occur with SARS-coronavirus, which, however, is very rare in the population (Table 5).

| **Pathogens from the *Coronovirideae* family** | **Cross-reactivity of primers and probes** |
|---|---|
| Human coronavirus 229E | excluded *in silico* |
| Human coronavirus OC43 | excluded *in silico* |
| Human coronavirus HKU1 | excluded *in silico* |
| Human coronavirus NL63 | excluded *in silico* |
| **SARS-coronavirus** | **≤ 80 %** |
| MERS-coronavirus | excluded *in silico* |
| **Other pathogens** | **Cross-reactivity of primers and probes** |
| Adenovirus (C1 and 71) | excluded *in silico* |
| Human Metapneumovirus (hMPV) | excluded *in silico* |
| Parainfluenza virus 1-4 | excluded *in silico* |
| Influenza A & B | excluded *in silico* |
| Enterovirus (for ex. EV68) | excluded *in silico* |
| Respiratory syncytial virus | excluded *in silico* |
| Rhinovirus | excluded *in silico* |
| *Chlamydia pneumoniae* | excluded *in silico* |
| *Haemophilus influenzae* | excluded *in silico* |
| *Legionella pneumophila* | excluded *in silico* |
| *Mycobacterium tuberculosis* | excluded *in silico* |
| *Streptococcus pneumoniae* | excluded *in silico* |
| *Streptococcus pyogenes* | excluded *in silico* |
| *Bordetella pertussis* | excluded *in silico* |
| *Mycoplasma pneumoniae* | excluded *in silico* |
| *Pneumocystis jirovecii* (PJP) | excluded *in silico* |
| *Candida albicans* | excluded *in silico* |
| *Pseudomonas aeruginosa* | excluded *in silico* |
| *Staphylococcus epidermis* | excluded *in silico* |
| *Staphylococcus salivarius* | excluded *in silico* |

*Table 5: In silico verification of cross-reactivity of primers and probes of SARS CoV-2 Direct LAMP 96-kit technology*

## Repeatability

The repeatability of the method was tested in 5 separate and independent experiments by measuring the same set of samples under the same conditions. Qualitative detection of the presence of SARS-CoV-2 virus was performed with 100% agreement and with an overall coefficient of variation of detection time in minutes (CV) of up **to 5%** (Table 6).

| sample | sample type | PCR result (Ct) | Test 1 | Test 2 | Test 3 | Test 4 | Test 5 | CV (%) | Conformity |
|---|---|---|---|---|---|---|---|---|---|
| | | | Detection time SARS-CoV-2 Direct LAMP 96-kit (min) | | | | | | |
| 1 | nasopharyngeal swab | positive (22.2) | 9.9 | 10.6 | 10.6 | 10.2 | 10.2 | **3 %** | 100 % |
| 2 | | positive (22.4) | 10.1 | 11.2 | 11.2 | 10.6 | 10.8 | **4 %** | 100 % |
| 3 | | negative | N | N | N | N | N | N/A | 100 % |
| 4 | | negative | N | N | N | N | N | N/A | 100 % |
| PC | positive control | x | 8.2 | 8.4 | 8.3 | 8.3 | 8.8 | **3 %** | 100 % |
| INT-STD | international standard (WHO 20/146) | x | 10.7 | 10.5 | 10.7 | 10.7 | 10.1 | **2 %** | 100 % |
| B.1.1.7 | RNA of British mutation B.1.1.7 | positive (10,5) | 14.1 | 13.7 | 14.1 | 14.0 | 14.5 | **2 %** | 100 % |

*Table 6: Repeatability of the assay evaluated by the CV of detection time in minutes. Determined CV was ≤ 5 %.*

## Robustness

The robustness of the assay was tested using a series of identical samples in 3 independent experiments. A defined change of conditions was made in each independent experiment. According to the results (Table 7) the SARS-CoV-2 Direct LAMP 96-kit was shown to give **100% identical results** with changes in the reaction conditions that were simulated:

1) lower reaction temperature (standard 65 °C, experimental 60 °C);

2) extending the time of thermal inactivation of the sample (standard 5 min, experimental 15 min);

3) change of the thermocycler (BioRad CFX96 vs. MIC).

| sample | sample type | PCR result (Ct) | Standard protocol | 1) Reaction temperature 60 °C | 2) Incubation time 15 min | 3) Change of the thermocycler | Conformity |
|---|---|---|---|---|---|---|---|
| | | | Detection time SARS-CoV-2 Direct LAMP 96-kit (min) | | | | |
| 1 | nasopharyngeal swab | positive (22.2) | 9.9 | 12.3 | 11.4 | 6.7 | 100 % |
| 2 | | positive (22.4) | 10.1 | 12.9 | 15.1 | 6.9 | 100 % |
| 3 | | negative | N | N | N | N | 100 % |
| 4 | | negative | N | N | N | N | 100 % |

*Table 7: Robustness of the method 100% with defined changes in experimental conditions compared to the standard protocol.*

## 14.2 CLINICAL FUNCTION

The diagnostic parameters of the BioVendor SARS-CoV-2 Direct LAMP 96-kit were evaluated by comparison with a commercially available CE-IVD RT-qPCR method on a set of 105 nasopharyngeal swab samples. The results of the Performance evaluation are shown in Table 8.

| Sample | PCR determination | Diagnostic sensitivity | Diagnostic specificity | Positive predictive value | Negative predictive value |
|---|---|---|---|---|---|
| | | SARS-CoV-2 Direct LAMP 96-kit | | | |
| nasopharyngeal swab (n = 105) | Ct < 35 | **88 %** | **100 %** | **100 %** | **100 %** |
| | Ct < 30 | **88 %** | | | |
| | Ct < 25 | **95 %** | | | |

*Table 8: Diagnostic accuracy of SARS-CoV-2 Direct LAMP 96-kit determined on clinical samples of nasopharyngeal swabs*

## 15. TROUBLE SHOOTING AND FREQUENTLY ASKED QUESTIONS

| No. | Problem | Possible cause | Solution |
|---|---|---|---|
| 1 | Detection of positive signal in NC tube.<br>The PC signal is correct (positive). | Test did not run correctly, and the results are not valid. The NC well or the entire reaction mixture was contaminated with SARS-CoV-2 virus. Contamination can come from one of the samples or from a PC. | Repeat the test with new reagents. Keep work clean and separate the preparation of samples and controls. |
| 2 | There is no signal detected in the PC tube and NC tube is positive. | Test did not run correctly, and the results are not valid. PC and NC could be interchanged. Material contamination or a technical problem could occured. | Repeat the test with new reagents. Check the device and make sure that the technical equipment is in correct settings. Record the test layout correctly. |
| 3 | There is no signal detected in the PC tube. The negative control tube is measured correctly (negative signal) | The test did not run completely correct, positive results for the samples can be considered as suspicious. The PC was not properly added to the test. Storage problem or another technical problem could appear. | Repeat the test with new reagents. Check the device and make sure that the technical equipment is in correct settings. In case of uncertainty, analyze the samples by another confirmatory method. |
| 4 | No positive signal in samples is detected. | If you detect a positive signal in PC and a negative signal in NC, all samples are evaluated as negative. If you do not detect a signal even for a PC, the test did not run correctly. | Check the PC result and repeat the test if necessary. |
| 5 | Resulting amplification curve from the thermocycler does not have the standard S shape. | This may be a reaction error. The result for the given sample(s) is invalid. | Repeat the analysis of given sample(s). |
| 6 | The sample result is negative, but the patient has symptoms of Covid-19 | The patient's viral load may be below the limit of detection of the assay. The patient may suffer another respiratory disease. | If there is uncertainty, analyze the patient sample using another confirmatory method (RT-qPCR). |

## 16. REFERENCES

**For more references to this product, visit our website www.biovendor.com**

[1] Coronavirus disease (COVID-19) Weekly Epidemiological Update and Weekly Operational Updates (available at https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports)

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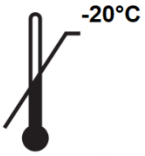
[4] DUCHENE, Sebastian; FEATHERSTONE, Leo; HARITOPOULOU-SINANIDOU, Melina; RAMBAUT, Andrew; LEMEY, Philippe; BAELE, Guy. Temporal signal and the phylodynamic threshold of SARS-CoV-2. Virus Evolution 2020-07-01 year. 6, n. 2 (available at: https://academic.oup.com/ve/article/6/2/veaa061/5894560)

[5] David Cyranoski, Alarming COVID variants show vital role of genomic surveillance, Nature 589, 337-338 (2021) (available at: https://www.nature.com/articles/d41586-021-00065-4)

## 17. SYMBOLS

| | |
|---|---|
| REF | Catalogue number |
| LOT | Batch code |
|  | Use by date |
|   | Upper limit temperature |
|  | Manufacturer |
|   | Read electronic instructions for use - eIFU |
|   | In vitro diagnostic medical device |
|   | The content is sufficient for 96 tests |

# 18. ATTACHMENTS

## Attachment 1 – example of the test layout

### Test layout for 96-well format

- Add samples to the empty wells.
- Pipette the PC as the last!

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | | | | | | | | | | | | |
| B | | | | | | | | | | | | |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | **NC** |
| H | | | | | | | | | | | | **PC** |



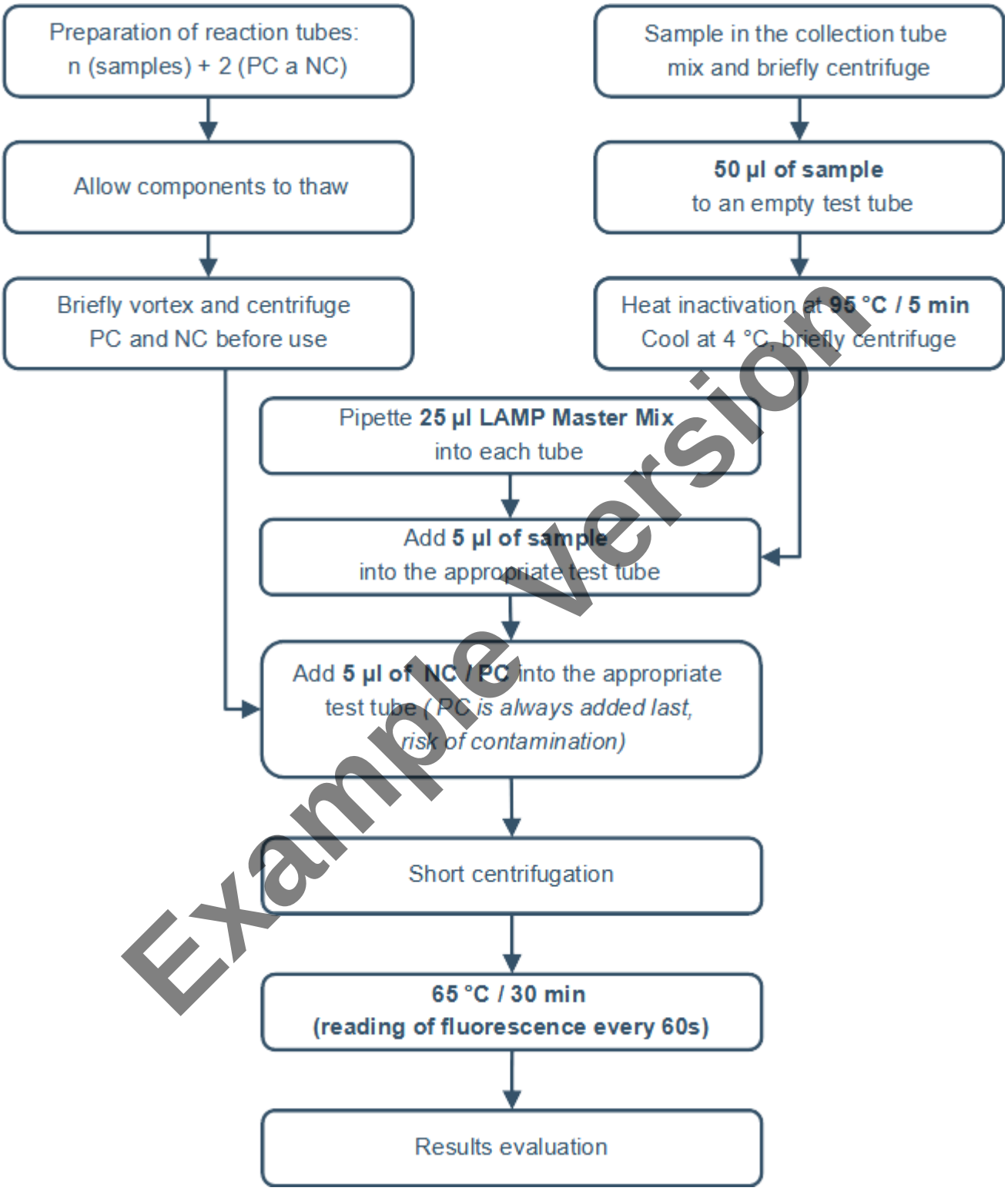
### Test layout for 48-well format

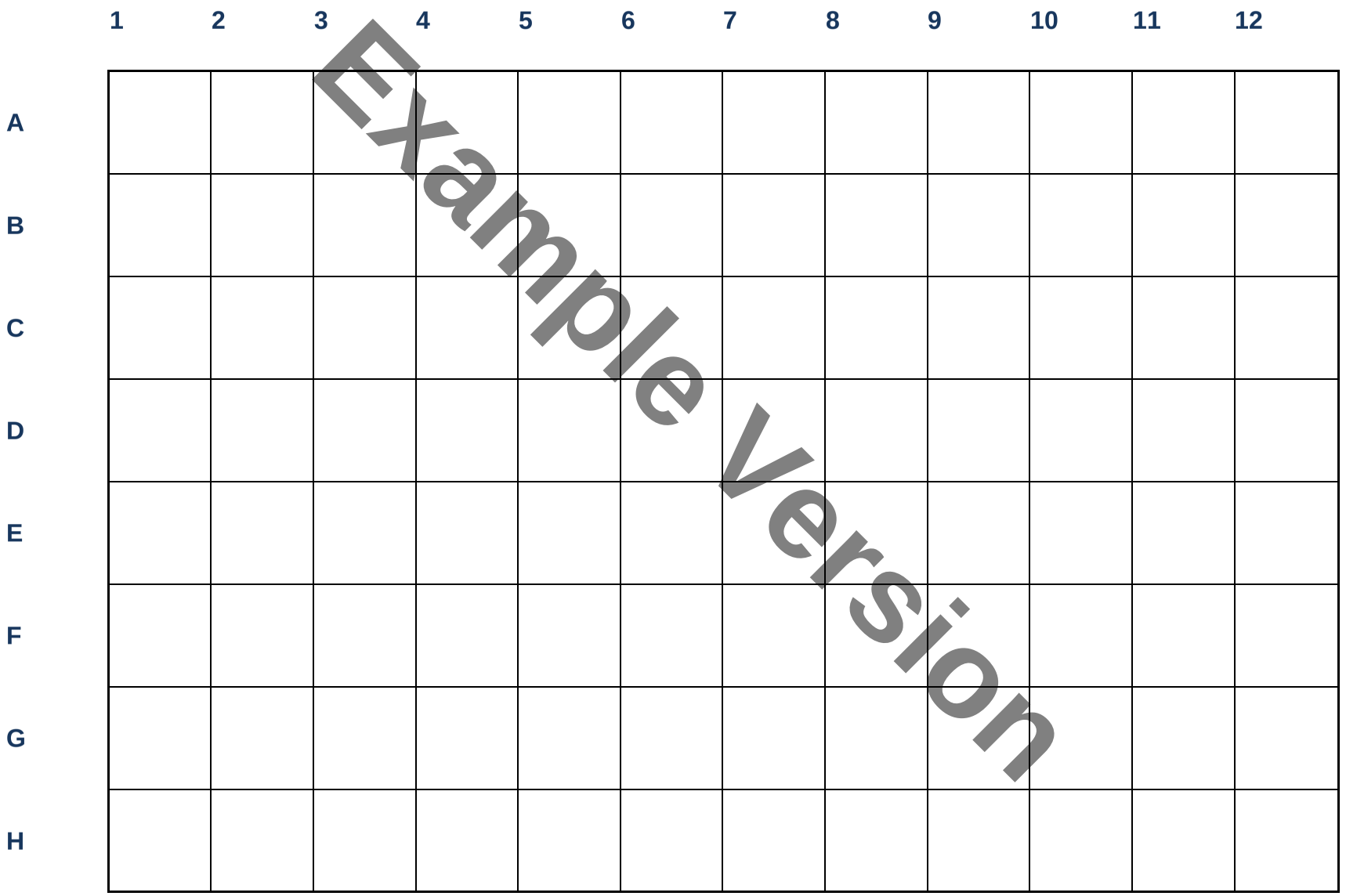
- Add samples to the empty wells.
- Pipette the PC as the last!

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| A | | | | | | |
| B | | | | | | |
| C | | | | | | |
| D | | | | | | |
| E | | | | | | |
| F | | | | | | |
| G | | | | | | **NC** |
| H | | | | | | **PC** |

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| A | | | | | | |
| B | | | | | | |
| C | | | | | | |
| D | | | | | | |
| E | | | | | | |
| F | | | | | | |
| G | | | | | | **NC** |
| H | | | | | | **PC** |

## Attachment 2 – Quick workflow summary





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