LAMPIGEN FLUOREMETRIC

SARS-CoV-2 RT-LAMP Kit

Instruction for Use (IFU)

In vitro RT-LAMP screening assay with fluoremetric detection for SARS-CoV-2 viral RNA;

Product numbers: LAMPPH2020-500,

LAMPPH2020-100

INTENDED TO USE

LAMPIGEN FLUOREMETRIC SARS-CoV-2 RT-LAMP Kit is intended to be used for detection of SARS-CoV-2 viral RNA in association with fluoremetric RT-LAMP devices (II, III and HT).

LAMPIGEN FLUOREMETRIC SARS-CoV-2 RT-LAMP Kit does not require RNA extraction and can be used directly on oropharyngeal / nasopharyngeal swab dilutions.

The causative agent of COVID-19, SARS-CoV-2 is an enveloped, positive sense RNA virus belonging to the Coronaviridae family. Regular and reliable detection of SARS-CoV-2 RNA is required to monitor the spread of the virus and for screening of clinical samples from patients displaying relevant symptoms of COVID-19.

LAMPIGEN FLUOREMETRIC SARS-CoV-2 RT-LAMP Kit is an in vitro diagnostic point of care test based on Reverse Transcription Loop-mediated isothermal AMPlification (RT-LAMP) technology for the detection of SARS-CoV-2 viral RNA. The detection is carried out in a one-step, closed tube format where the reverse transcription and subsequent amplification of the specific target sequence occur in the same reaction well. The Genie® HT device detects amplified product in real-time using fluorescence detection. It automatically run an anneal curve at the end of amplification, where the reaction is heated to 98°C and slowly cooled. This acts as a secondary confirmatory check - ensuring LAMP amplicons are specific to SARS-CoV-2. The final result is interpreted and reported automatically from both the amplification and the anneal temperature.

PRODUCT COMPONENTS & MATERIALS SUPPLIED

The kit is supplied in liquid form. A mastermix is supplied along with two sets of separate primer mixes representing two different genes (N gene and ORF1ab gene), both sufficient to run 500 reactions (Table 1, 2). Samples should be tested in duplicate, i.e. two reactions per sample

(Table 1): LAMPIGEN FLUOREMETRIC SARS-CoV-2 RT-LAMP Kit components stored at -17°C to -25°C

LAMPIGEN FLUOREMETRIC SARS-CoV-2 RT-LAMP Kit components			
Component	Number of vials	Reactions per vial	Lid colour
RT-LAMP Mastermix	1	1,000	white
Primer Mix – 1 (N gene)	1	500	Blue
Primer Mix – 2 (ORF1ab gene)	1	500	Red
Positive Control	1	20	
Negative Control	1	20	

(Table 2): LAMPIGEN FLUOREMETRIC SARS-CoV-2 RT-LAMP Kit components stored at room temperature

Component	Number of sets	Numbers per set	Total units
Tube-Strip	128	8	1,024
Virus Transport Medium (VTM)			500
Oro/nasopharyngeal swabs			500

All of the Fluoremetric RT-LAMP instruments operate with the OptiGene strip-of-8 reaction tubes. This proprietary consumable incorporates attached, locking caps which allow for a closed tube assay to prevent cross-contamination.

MATERIALS NEEDED BUT NOT SUPPLIED IN KIT

The following list (Table 3) includes materials and equipment that are required for use but are not included within the LAMPIGEN FLUOREMETRIC SARS-CoV-2 RT-LAMP Kit

(Table 3): Additional material and equipment required

Adjustable callibrated pipettes Pipette tips (filter tips) Mini vortex

Mini strip centrifuge
Genie® II, III or HT device
Genie® Strip Holder or cool block
Plastic wear
DNA/RNA degradation solution
Nuclease free water
Disinfectant
PPE
RNA extraction kits (just in case of inconclusive result)

LAMPIGEN FLUOREMETRIC SARS-CoV-2 RT-LAMP Kit tests have been validated for use on Fluoremetric RT-LAMP devices (Genie® devices; the OptiGene Limited) (Table 4). These devices are manufactured by OptiSense Limited of Horsham, West Sussex UK.

Table 4. Fluoremetric RT-LAMP devices (Genie® devices)

Genie® III
• Number of wells: 8 wells (1 x Genie® strip)
• Dimensions: 25 (L) x 16 (W) x 8.5 (H) cm
• Rechargeable lithium battery
Genie® II
• Number of wells: 16 wells (2 x Genie® strips)
• Dimensions: 20 (H) X 21 (D) X 30 (W) cm
• Rechargeable lithium battery
Genie® HT
• Number of wells: 96 wells (12 x Genie® strips)
• Dimensions: 63.5 (L) X 43.4 (W) X 15.3 (H) cm

All of the Genie® instruments should be set up and run by following the instruction manual provided.

SAMPLE COLLECTION AND HANDLING

LAMPIGEN FLUOREMETRIC SARS-CoV-2 RT-LAMP KIT includes swabs for nasal specimen collection.

Swab should be inserted first into one nostril of the patient up to 2.5 cm (1 inch) from the edge of the nostril. It must be rolled 5 times along the mucosa inside the nostril to ensure that both

mucus and cells are collected. Then using the same swab process should be repeated for the other nostril to ensure that an adequate sample is collected from both nasal cavities. Later the sawb must be withdrawn from the nasal cavity. The sample is now ready for processing using LAMPIGEN FLUOREMETRIC SARS-CoV-2 RT-LAMP KIT.

SAMPLE PREPERATIONS & PROCEDURES

Testing for the presence of SARS-CoV-2 RNA should be performed in an appropriately equipped facility and by trained staff. All samples should be handled as if they are infectious, following conventional biosafety precautions. A unidirectional workflow should be implemented in seperate working areas where RT-LAMP reaction set up, sample preparation and amplification are done.

This protocol describes the procedure for using the LAMPIGEN FLUOREMETRIC SARS-CoV-2 RT-LAMP Kit assay for the detection of SARS-CoV-2 directly from swabs. This method, when combined with the direct use of swabs, is to be utilised as a screening and diagnostic test to identify strong positive samples. The steps are as follows:

1) Reaction mix preparation (in the clean workspace):
    a. Wipe surfaces and pipettes with DNA/RNA degradation solution.
    b. Ensure the RT-LAMP Mastermix and Primer Mixes are vortexed well before use. A fresh reaction mix should be prepared before each batch of samples is tested. The time which reactions are at room temperature should be minimised. We recommend reactions are set up using a cooled block, for example Genie® Strip Holders. (Table 5.)

Table 5. Reaction mix preparation

Reagents	Volume per reaction (µl)
RT-LAMP Mastermix	17.5
Primer Mix - 1	2.5
Primer Mix - 2	2.5

    c. The time which reactions are at room temperature should be minimised. We recommend reactions are set up using a cooled block, for example Genie® Strip Holders.
    d. After briefly vortexing, aliquot 20 µl of the prepared reaction mix into each required Genie® tube.
    e. For the NTC: Add 5 µl nuclease free water to the NTC reaction and close the lid to the locked position. Ensure the reaction is well mixed.

2) Addition of the sample (in the swab processing workspace):
   a. Add 17,5 µl of mastermix to two adjacent Genie® tubes. Then add 2,5 µl of primer mix-1 into the first tube and 2,5 µl of primer mix-2 into the second tube. Samples should be tested in duplicate: wells 1&2; wells 3&4; wells 5&6; wells 7&8. Duplicates must be set up in the configuration shown in Figure 1, 2.

Figure 1: Preperation of samples.

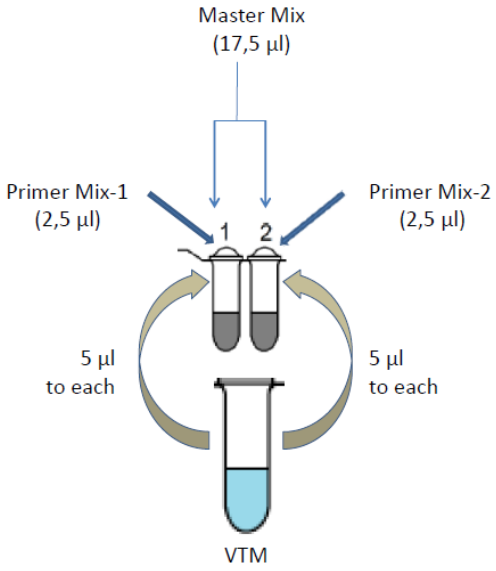
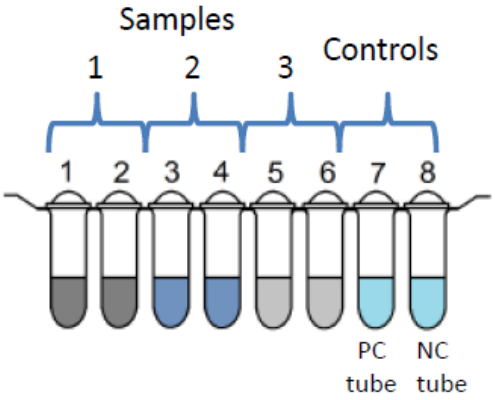


Figure 2: RT-LAMP duplicate layout. Duplicates should be run in wells 1&2; wells 3&4; wells 5&6; wells 7&8.



   b. Genie® platforms analyse duplicates according to this set up (Figure 2). Ensure the reactions are well mixed.
   c. Only open the lids of Genie® tubes for one sample at a time; keep the others loosely closed until required. Close to the locked position after addition of each individual

sample of 5 µl from VTM . The time which reactions are at room temperature should be minimised. We recommend samples are added to reactions within a cooled block, for example Genie® Strip Holders.

d. Disinfect the Genie® tubes (ensure each tube is fully locked, spray with disinfectant and dry with a paper towel) before removing from the sample processing workspace. Take these tubes to the amplification workspace.
e. The Genie® tubes must NOT be opened after removal from the swab processing workspace.

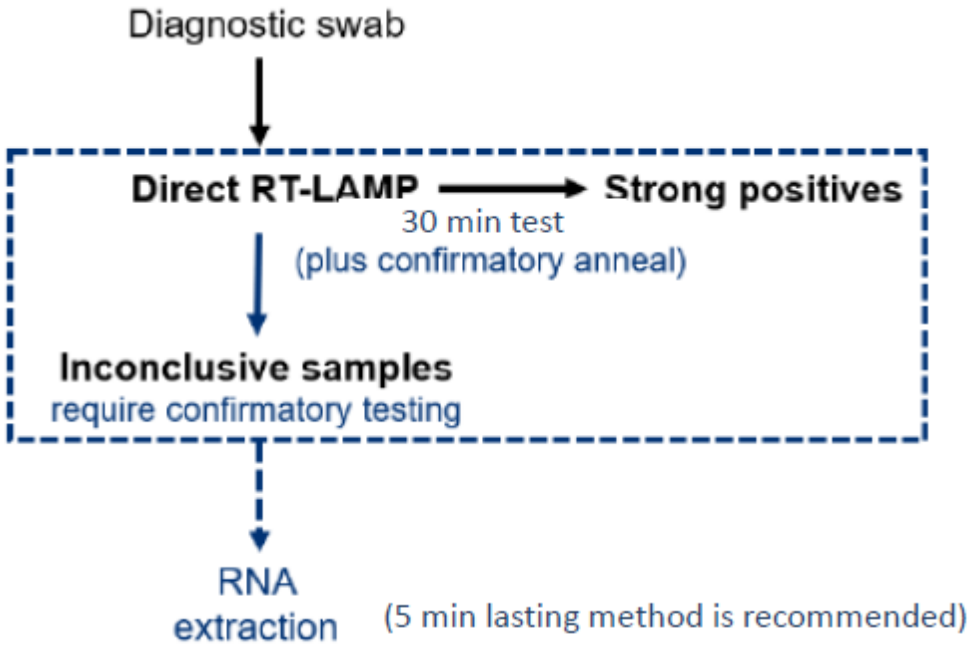
3) Setting up the Genie® (in the amplification workspace):
    a. Please refer to the instrument manual for full details.
    b. Turn on the Genie® II/III or HT machine at the main switch and wait for the software to initialise.
    c. Ensure the Genie® strips are dry and free from disinfectant before loading onto the machine. Additionally, ensure the liquid is at the bottom of the tube and there are no bubbles by flicking the tubes carefully, or spinning in a Genie® strip microcentrifuge.
    d. Load each Genie® strip into the chosen heat block.
    e. Follow the screen's instructions, start the test and enter the relevant sample details for each Genie® tube. For the Genie® II and HT, heat blocks are random access and can be used independently of one another.

4) Interpretation of Results
    a. Genie® software will automatically analyse results and report samples as SARS-CoV-2-positive (SARS-CoV-2 RNA detected) or state that confirmatory testing is required (levels of SARS-CoV-2 RNA below the detection limit of the assay)
    b. An algorithm on the Genie® platforms analyses both the amplification plot and the anneal temperature to determine SARS-CoV-2-positive and confirmatory testing required reactions. A SARS-CoV-2-positive is reported automatically if (i) the fluorescence level of the amplification plot rises above a defined threshold and (ii) the peak of the anneal first derivative is above a defined threshold and lies within a specified temperature range.
    c. The results of each run are automatically saved with a unique run number ID and are stored by day and month.
    d. For the Genie® HT, the last two digits after the hyphen represent the heat block that the run was performed on.
    e. The detection is carried out in a one-step, closed tube format where the reverse transcription and subsequent amplification of the specific target sequence occur in the same reaction well. The Genie® HT device detects amplified product in real-time using fluorescence detection. It automatically run an anneal curve at the end of amplification, where the reaction is heated to 98°C and slowly cooled. This acts as a

secondary confirmatory check - ensuring LAMP amplicons are specific to SARS-CoV-2.

f. It is recommended each batch of samples include at least one NTC
g. In Genie II devices, 3 samples (by examing the double genes) and 1 paired of controls (positive and negative) are tested. On the other hand, Genie HT can provide testing up to 47 samples (with 2 genes) including with both controls (NC and PC).
h. If an inconclusive result happens RNA extraction is required for confirmatory testing (Figure 3). In that case, a fast RNA extaction method which lasts 5 minutes is highly recommended.

Figure 3: Confirmatory Testing Requirement in case of inconclusive sample



5) Confirmation of negative results

LAMPIGEN FLUOREMETRIC SARS-CoV-2 RT-LAMP Kit, when combined with the direct use of swabs, is to be utilised as a screening test to identify strong positive samples. Consequently, samples which display no amplification need to be subjected to confirmatory testing, such as the gold-standard qRT-PCR (or RT-LAMP) following RNA

extraction. Please choose an appropriate CE IVD extraction methodology and CE IVD approved molecular test to perform the confirmatory testing.

6) Limitations of the Test

LAMPIGEN FLUOREMETRIC SARS-CoV-2 RT-LAMP Kit has been validated for use with oropharyngeal / nasopharyngeal swab samples. In house validation should be performed if using a different sample type.

• Assay validation has been performed using Genie® platforms only.

• Procedures in this IFU must be followed; any deviations may result in assay failure or cause erroneous results.

• Test quality is dependent on the quality of the sample.

• All results should be interpreted by a health care professional in the context of medical history, clinical symptoms and other diagnostic tests.

• Very low levels of RNA target, below the limit of detection, might be detected but results may not be reproducible.

• LAMPIGEN FLUOREMETRIC SARS-CoV-2 RT-LAMP Kit is specific to SARS-CoV-2, as such these RT-LAMP kits cannot rule out diseases caused by other pathogens.

• LAMPIGEN FLUOREMETRIC SARS-CoV-2 RT-LAMP Kit is intended as a screening tool to identify strong positive samples; a negative result does not rule out the possibility of infection.

• Interpretation of results should account for the possibility of false negative and false positive results (Table 6).

Table 6. Potential causes of false negative and false positive results

False negative results	False positive results
Improper collection, handling and/or storage of samples	Improper handling of samples and/or positive controls
Samples with a viral load below the limit of detection	Contamination of workspaces
Improper sample processing (including unsuitable extraction kit)	Opening of reactions post-amplification
Mutations or polymorphisms in primer or probe binding regions	Deviation from handbook protocol
The presence of RT-LAMP inhibitors or interfering substances	
Deviation from handbook protocol	

7) Quality Control

In accordance with Pharmaline Sağlık Hizmetleri Tic. A.Ş. (ISO 13487:2016) Quality Management System, each component of the LAMPIGEN FLUOREMETRIC SARS-CoV-2 RT-LAMP Kit is tested against predetermined specifications to ensure consistent product quality.

8) Performance Evaluation

1. Validation Results

The diagnostic sensitivity and specificity were determined using our panel (Table 7)

Table 7. Diagnostic sensitivity and specificity of LAMPIGEN SARS-CoV-2 RT-LAMP

	Direct RT-LAMP (Duplicates)*
% Detection of samples with CT <25a (Very high viral load; high risk of shedding) (25 qRT-PCR positive samples)	100%
% Detection of samples with CT <30a (41 qRT-PCR positive samples)	80%
% Detection of samples with CT <33a (44 qRT-PCR positive samples)	75%
% Detection of all qRT-PCR positives (CT <40)a (49 qRT-PCR positive samples)	67%
Overall Diagnostic Sensitivity (Se) (Concordance with RT-qPCR to detect positives)	100% (samples with <CT25) 80% (samples with <CT 30) 75% (samples with <CT33) 67% (samples with <CT40)
Overall Diagnostic Specificity (DSp)a (Concordance with RT-qPCR to detect negatives)	97%a
Analytical Specificity for SARS-CoV-2 (ASp)**	100%
With confirmatory testing of Direct RT-LAMP "negatives"	Se 99.95% Sp 98.40%.

a Samples were tested in singles using the qRT-PCR. For the statistics, it was assumed that the qRT-PCR results were correct. Cycle threshold (CT) is the number of cycles required for the fluorescent signal to cross a defined thureshold for the comparator qRT-PCR.

* For duplicates: performance of LAMP when testing samples in duplicate and classifying a sample as positive when at least one of the duplicates is LAMP positive.

** Analytical specificity was determined using a panel of respiratory pathogen including other common coronaviruses.

2. Analytical Specificity

Analytical specificity was determined using a panel of respiratory pathogens (Table 8).

Table 8. Analytical specificity of LAMPIGEN SARS-CoV-2 RT-LAMP

Respiratory Pathogen	Result of LAMPIGEN SARS-CoV-2 RT-LAMP Kit
Legionella pneumophila	Negative
Haemophilus influenzae	Negative
Streptococcus pneumoniae	Negative
Bordetella parapertussis	Negative
Human Coronavirus NL63	Negative
Human Coronavirus 229E	Negative
Human Coronavirus OC43	Negative
Human Coronavirus HKU1	Negative
Influenza A virus (Flu A)	Negative
Influenza B virus (Flu B)	Negative
Influenza A/H1	Negative
Influenza A/H3	Negative
Human Parainfluenza 1	Negative
Human Parainfluenza 2	Negative
Human Parainfluenza 3	Negative
Human Parainfluenza 4	Negative
Human bocavirus	Negative
Respiratory syncytial virus	Negative
Human adenovirus	Negative
Enterovirus	Negative
Parechovirus	Negative

WARNINGS AND PRECAUTIONS

All samples should be handled as if they are infectious, following conventional biosafety precautions. National guidelines on biosafety should be followed in all circumstances.

Testing for the presence of SARS-CoV-2 RNA should be performed in an appropriately equipped facility and by trained staff.

KIT STORAGE AND STABILITY

LAMPIGEN SARS-CoV-2 RT-LAMP Kit assays are shipped cold. On arrival, LAMPIGEN SARS-CoV-2 RT-LAMP Kit assays should be stored in the original packaging at -17°C to -25°C (NOT using a frost-free freezer). The kits should not be used past the expiry date as indicated on the outer packaging label, Direct RT-LAMP Mastermix and 10X COVID-19 Primer Mix tube labels. Keep Direct RT-LAMP Mastermix away from light. Reagents may be aliquoted into smaller volumes if necessary.

ASSISTANCE

If you have a question regarding the use of this product, please contact us.

Phone Number: +90 216 346 86 66 pbx

REFERENCES

World Health Organization (21 January 2020). Situation report – 1: Novel Coronavirus (2019-nCoV). Available at https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200121-sitrep-1-2019-ncov.pdf?sfvrsn=20a99c10_4

2) World Health Organization (31 January 2020). Situation report – 11: Novel Coronavirus (2019-nCoV). Available at https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200131-sitrep-11-ncov.pdf?sfvrsn=de7c0f7_4

3) Fowler et al. (2020). A reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assay for the rapid detection of SARS-CoV-2 within nasopharyngeal and oropharyngeal swabs at Hampshire Hospitals NHS Foundation Trust. medRxiv, pre-print. doi: https://doi.org/10.1101/2020.06.30.20142935.

TRADEMARKS