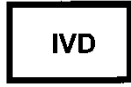


AUMED test RT - LAMP Assay SARS-CoV2



Instructions for use of the kit

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1. NAME OF THE KIT:

AUMED test RT-LAMP Assay SARS-CoV2- kit for RT-LAMP detection of SARS-CoV-2

2. USE

The kit is intended for diagnosis of the presence of SARS-CoV-2 virus in isolated viral RNA samples - for 45 samples.

3. PRINCIPLE OF THE TEST:

Molecular amplification technique RT-LAMP (Reverse transcription - Loop mediated isothermal amplification) allows the amplification of nucleic acids with high specificity, efficiency and speed under isothermal conditions – i.e. at a constant temperature, achievable in incubators, hybridization ovens or dryers. The results are reliably read based on the color change with the naked eye.

The test involves the detection of SARS-CoV2 virus and the detection of an internal control (IC) to check the correct collection and processing of the sample.



4. KIT CONTENTS:

- Reagent box for 45 tests

Content	Volume (ul)	Color	KS
MasterMix SARS-CoV2	1470	Orange	1
MasterMix IC	1470	Green	1
EnzymeMix	320	Black	1
SARS-CoV2 PC	15	Red	1
IC PC	15	Blue	1
Nuclease-free Water	1800	White	1

IC = internal control

PC = positive control

- A bag containing a 96-hole PCR plate and a plate of colored caps in a light-tight package. Should be stored at room temperature, caps in the original light-tight package or in the dark.

5. MATERIAL REQUIRED - NOT SUPPLIED WITH THE KIT:

- Automatic and multichannel pipettes
- Appropriate reservoir for the reaction mixture
- Pipette tips with RNase and DNase free aerosol filters
- Plate incubation device at 63 °C

6. WORKING PROCEDURE FOR DETERMINING THE PRESENCE OF SARS-COV2 VIRUS:

- Preparation of the test must be carried out on ice or in a refrigerated stand at 4 °C
- Master mixes and water thawed and place into refrigerated stand
- For each sample is performed separately **one response to the presence of SARS and one internal control (IC)**
- To check the progress of the SARS and IC reactions the positive and negative controls are included
- To detect SARS and IC, prepare reaction mixtures according to the following table
 - Attention, the total volume is greater than 2 ml!

	50 reactions *	1 reaction
Master Mix	1350 ul	27 ul
Enzyme Mix	150 ul	3 ul
water	750 ul	15 ul
total	2250 ul	45 ul

* 45 samples + PC + NC + 3 extra reactions for pipetting losses

- the PCR plate place in a refrigerated stand



- Pipette the SARS mix - use 45ul into 47 holes in a plate (preferably from a reservoir with a multichannel pipette)
 - Add 5ul of SARS-CoV2 PC positive control to hole A1
 - Add 5ul of water to hole B1 = negative control NC
 - Pipette 5ul of samples into another 45 holes
 - Mix the contents gently by drawing repeatedly into the pipette
- Pipette the IC mix - use 45ul into 47 holes in a plate (preferably from a reservoir with a multichannel pipette)
 - Add 5ul of IC PC positive control to hole A7
 - Add 5ul of water to hole B7 = negative control
 - Pipette 5ul of samples into another 45 holes
 - Mix the contents gently by drawing repeatedly into the pipette
- Carefully close the test tubes with the prepared color caps so that the reaction mixture does not come into contact with the cap. Seal well.
- Place the PCR plate into an incubator preheated to 63 °C and incubate for 55 minutes.
 - Handle the plate carefully so that the reaction mixture does not come into contact with the cap!
- Remove the PCR plate from the incubator and cool down for 5 minutes at room temperature.
- Press the caps on the plate again.
- Turn the plate upside down and tap the reaction mixture into the cap with a quick motion.
- Repeat the tapping to make sure that all test tubes in the plate have colored (the reaction mixture must remain in the caps for at least 2 minutes).
- Negative samples and negative controls discolor after approximately two minutes.
- **Never open the tubes!**

7. EVALUATION:

Samples must be evaluated within the next 2 hours.

Never open the tubes!

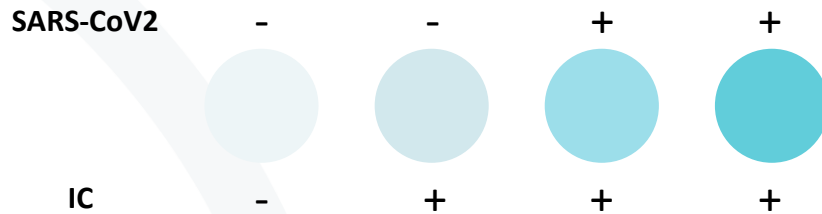
A) With the naked eye

- Tap the reaction mixture into the tubes and carefully rotate the plate with the caps down so that the reaction mixture remains at the top of the tube tips.
- Place the enclosed paper template on the plate so that the sample positions are correctly marked.
- The positive control must be blue and the negative control colorless.
- Read individual samples according to the calibrator.



- For the IC internal control, samples must be positive. If the sample is negative, even though the control is OK, then the sample has been poorly taken or processed and a new sample must be taken.

Calibrator for determination of positive (+) and negative (-) samples. Positive samples in IC have a weaker shade of blue than SARS-CoV2 samples.



b) Digital photo documentation

- **PCR plate**
 - Tap the reaction mixture into the tubes and carefully rotate the plate with the caps down so that the reaction mixture remains at the top of the tube tips.
 - Place the enclosed paper template on the plate so that the sample positions are correctly marked.
 - Remove the paper template and photograph the plate with the tubes facing upwards with a mobile phone, digital camera or photo documentation device.
- **PCR strip**
 - Tap the reaction mixture into the tubes and carefully rotate the strip with the caps down so that the reaction mixture remains at the top of the tube tips.
 - Place the plastic template on the strip so that the first tube is in position A1
 - Remove the paper template and photograph the plate with the tubes facing upwards with a mobile phone, digital camera or photo documentation device.
 - Photograph the entire plastic strip template in this position with a mobile phone, digital camera or photo documentation device.
- Take the photo in daylight or artificial lighting, on a white background, or on a light table.
- Take a photo using the automatic settings of the digital device.
- Copy the photo to a PC and save it as a .jpg file.
- Graphical analysis is performed using the Protean-LAMP96 script.

8. WARNING:

- Reactions and reading of results must be performed in areas physically separate from the test preparation areas.
- After the reaction at 63 °C, **the test tubes with reaction mixture MUST NOT be opened under any circumstances!** After reading the results, the test tubes should be discarded as soon as possible in accordance with local regulations.
- Reagents are stored at -20 ± 5 °C, plates with caps at room temperature in dry in light-tight and airtight packaging.
- The safety regulations must be observed when working.

9. PROBLEMS SOLVING:

- If, as a result of careless handling, the reaction mixture comes into contact with the color in the cap before or during the reaction, the whole test must be repeated.
- If the positive control is not blue, an error occurred during the test and the test must be repeated.
 - Possible errors: poorly mixed reaction mixture, not using a refrigerated stand, omission of the positive control, insufficient temperature for the reaction
- If the negative control is blue, either contamination or addition of a positive control or sample has been added to the negative control hole

10. SAFETY REGULATIONS:

All components of the kit are for laboratory use only.

Do not eat, drink or smoke while working. Wear protective gloves and wash your hands thoroughly after work. Be careful not to spill specimens or form an aerosol.

11. RESPONSIBILITY:

- 1) The manufacturer guarantees the usability of the kit as a whole.
- 2) The manufacturer accepts no liability for non-conformities caused by incorrect use of the test.
- 3) Follow the instructions in the manual exactly. Non-reproducible results may arise in particular:
 - * insufficient mixing of reagents and samples before use
 - * inaccurate pipetting and non-compliance with incubation time and temperatures
 - * using the same tip when pipetting different solutions or swapping caps


12. STORAGE AND EXPIRATION


- Store the reagent box at -15 to -25 °C. The expiration date of the kit is indicated on the outer package.




- Store the bag containing the 96-hole PCR plate and the plate of colored caps at room temperature, in the original light-tight container or in the dark. The expiration date is stated on the outer label.

13. USED SYMBOLS

 Conformité Européenne – the product meets EU legislative requirements

 Diagnostics *in vitro*

 Expiration

 Batch reference number

Date of the latest version of this manual: 12/2020

