

*abTES*<sup>™</sup> COVID-19 LAMP-C kit

## Fast Detection of 2019 Novel Coronavirus (COVID-19) by LAMP technology

### Instruction for Use

## *abTES*<sup>™</sup> COVID-19 LAMP-C Kit

### Kit Version: 1.0



300145 (50 Reactions)  
300146 (100 Reactions)



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Store at -25 °C to -15 °C



Research use only

For use on heat block, water block, thermocycler or instrument-appropriate reaction vessels.

### 1. Pathogen Information

Coronavirus disease 2019 (COVID-19) is a disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)<sup>4</sup>. SARS-CoV-2 is a single-stranded, positive-sense RNA virus which is capable of person-to-person transmission. COVID-19 has spread to more than 20 countries within a month it was declared a pandemic on March 11, 2020<sup>1</sup>. It is genetically highly related to SARS and MERS coronaviruses<sup>2</sup>.

So far, seven coronavirus species including COVID-19 are known to infect human. Four viruses, including 229E, OC43, NL63 and HKU1, typically cause a mild cold. However, people infected with the other three, including SARS, MERS and COVID-19, may develop acute and severe respiratory diseases, fever, cough and even death<sup>3</sup>.

### 2. Test Description

The *abTES*<sup>™</sup> COVID-19 LAMP-C kit is based on loop-mediated isothermal amplification (LAMP) technology, which enables rapid amplification of COVID-19 within 45 min, using basic lab equipment such as water bath, heating block, etc.

The kit contains all the necessary reagents for specific detection using multiple target-specific primers.

### 3. Storage Conditions



#### IMPORTANT!

- Improper storage conditions may compromise product performance.
- Do not exceed three freeze-thaw cycles.

The components of *abTES*<sup>™</sup> COVID-19 LAMP-C Kit should be stored in the dark, at -20°C in a **NON**-frost-free freezer. Frost-free freezers go through freeze-thaw cycles to remain frost-free and may cause accelerated degradation of enzymes and nucleic acids. Avoid repeated thawing and freezing (max 3 times) as this may lower the sensitivity. If reagents will be used intermittently, it is suggested to keep the reagents frozen in aliquots.

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## 4. Kit Components

Table 1. abTES™ COVID-19 LAMP-C Kit components.

Tubes	Components	Volume	
		300145 (50 rxn)	300146 (100 rxn)
1	Master Mix	1x 500 µL	2x 500 µL
2	Primer Mix	1x 250 µL	2x 250 µL
3	COVID-19 Positive Control	1x 100 µL	1 x 100 µL
4	Nuclease-free Water	1x 100 µL	2x 100 µL
5	Mineral oil	1x 500 µL	2x 500 µL

## 5. Materials and Devices Required but not Provided

- Benchtop centrifuge with a rotor for 2 ml tubes
- Vortex mixer
- Disposable powder-free gloves
- Nucleic acid extraction kit
- Pipettes (adjustable) and pipette tips with filter (disposable)
- Desktop centrifuge with rotor
- Water bath / heat block with constant temperature
- Clear 96-well plate/ clear PCR tube/ clear microcentrifuge tube

**NOTE: qPCR consumables must be certified free from DNA, DNase, RNase and PCR inhibitors**

- 96-well PCR plate clear sealing film/ cap
- Ice box/cooling block
- Ice

## 6. General Precautions

- Proper aseptic technique should always be used.
- Always work in RNase-free environment.
- Do not exceed three freeze-thaw cycles.
- Do not use the kit after its expiration date.
- Improper storage conditions may compromise product performance.
- Wear disposable gloves, laboratory coats and eye protection when handling samples and reagents. Wash hands thoroughly thereafter.
- Highly recommended to use disposable pipette tips with filter.
- Always select the pipette with the lowest volume possible and the matching filter tip
- Always treat samples as biohazardous and infectious.
- Decontaminate and dispose of all potentially infectious materials in accordance with local and national regulation.
- Regular decontamination of setup locations and equipment using 1% bleach is recommended to prevent potential carryover contamination.

## 7. Contamination and Inhibition

- The presence of inhibitors may lead to invalid or false-negative results
- If sample preparation system containing ethanol, make sure any traces of ethanol is eliminated. Ethanol is a strong inhibitor.
- Do not open reaction tubes/plates after amplification to avoid amplicon contamination.
- Store positive materials (test samples, controls, and amplicons) separately from all other reagents and add to the reaction mix in a separate facility.
- Use sterile pipette tips with filters and replace the tip for every procedure.
- Do not interchange tube as this may lead to cross-contamination.
- Laboratory area can be contaminated with amplicon or test sample if the waste materials are not carefully handled and disposed.

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## 8. Procedures

**IMPORTANT!**

- Be sure read section 6 and 7 before use.

## 8.1. Nucleic Acids (NA) Extraction

**IMPORTANT!**

- If sample preparation system containing ethanol, make sure any traces of ethanol is eliminated. Ethanol is a strong qPCR inhibitor.

Standard NA extraction kits are compatible with this assay but must be validated by the user. Please carry out NA extraction as per instructed in the manufacturer's extraction kit manual.

## 8.2. Reaction Setup

**IMPORTANT!**

- Only use extracted and purified sample.
- Proper aseptic technique should always be used.
- Always work in RNase-free environment.
- Do not exceed three freeze-thaw cycles.
- Do not use the kit after its expiration date.
- Before starting an assay, thaw all the components thoroughly at room temperature
- Always keep samples and components on the ice during use.
- When the components are thawed, mix the components and centrifuge briefly.
- Always include at least one positive and one negative controls on each run.
- Run sample immediately after PCR reaction setup to prevent degradation of RNA samples.
- Always select the pipette with the lowest volume possible and the matching filter tip
- Salt may appear the bottom of the master mix, mix thoroughly every time before use.
- The colour of master mix might turn from pink to orange, this will not affect the performance.
- If non-PCR equipment to be used for LAMP reaction, please load 8-10ul of mineral oil to cover the reaction mix to avoid evaporation. Do not mix.

1. Thoroughly thaw all components, mix, and spin briefly. Keep all thawed components and samples on ice.
2. Prepare your PCR reaction based on the following pipetting scheme:

**Table 2. Sample reagent preparation calculation for one reaction.**

Reagent	Volume per reaction		
	Test sample reaction	Positive control reaction	Non template control reaction
Master Mix	10 µL	10 µL	10 µL
Primer Mix	5 µL	2 µL	2 µL
Nuclease-Free Water	-	-	5 µL
Positive Control	-	5 µL	-
Extracted Test Sample	5 µL	-	-
<b>Total Volume</b>	<b>20 µL</b>	<b>20 µL</b>	<b>20 µL</b>

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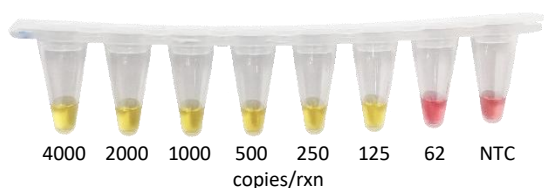
3. Mix the reaction mix thoroughly and spin briefly. Dispense 15µl of the reaction mix into each required well of a 96- well plate or each required PCR tube.
4. Add 5µl of test sample or positive control into each required well of a 96-well plate or each required PCR tube.  
**Always include at least one positive and one negative controls on each run.**

**If non-thermocycler equipment to be used for LAMP reaction, please load 8-10ul of mineral oil to cover the reaction mix to avoid evaporation. Do not mix.**

5. Seal all wells or tubes tightly. Mix the reaction mix by flipping or gently vortex. Centrifuge briefly to settle tube contents and eliminate large bubble.
6. Incubate at 63 °C for 45 minutes. **After 45 min, the reaction vessels can be examined. If the color change is not robust, incubate at 63 °C for another 15 min.**
7. After the run is completed, it is advised to place reaction vessels **on ice for 15-30 seconds**. This will intensify the color.

**8.3. Performance Characteristics**

Based on the preliminary data, the limit of detection (LOD) of this assay was about 25 copy/µl (125 copies/ rxn).

**8.4. Data Analysis and Interpretation****IMPORTANT!**

- **abTES™ COVID-19 LAMP-C Kit is function well if positive control turns from pink to yellow/orange colour.**

Remove tubes from incubation and examine by eye. Detected reactions will turn yellow/orange while negative reactions will turn pink. If the color change from positive control is not robust, return reactions to 63 °C for additional 15 mins. Reactions can be examined earlier if desired, e.g. high copy reactions can exhibit color change in 30 mins. Color change can be intensified by allowing reaction to cool to room temperature



Yellow/orange: Detected

Pink: Undetected

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## 9. Troubleshooting

Table 7. Example of unexpected observations on positive control, non template control and test sample.









Sample	Observation	Potential Cause	Solution
<b>Positive control</b>	No colour change (remain in pink colour)	<ul style="list-style-type: none"> <li>• Check programmed temperature settings against the protocol given.</li> <li>• Affirm that proper storage was done and check the expiry date on the kit.</li> <li>• Check the proper volume of the reagents added during the setup.</li> <li>• Positive control is not added</li> <li>• Positive control is added into the wrong tube</li> <li>• Inhibitors present in test sample</li> <li>• Interference from test sample containing some impurities</li> <li>• Interference from substances present inside tubes/strips</li> </ul>	<b>Observation no.: 1 and 2</b> <ul style="list-style-type: none"> <li>• Do not use expired kit</li> <li>• Store kit at -25°C and -15°C</li> <li>• Check and rerun with correct cycling condition</li> <li>• Refer section 8.2 for reaction setup</li> <li>• Ensure sample is assigned accordingly</li> </ul>
<b>Non template control</b>	Colour changed from pink to yellow/orange	<ul style="list-style-type: none"> <li>• Contamination from either extracted sample and/or positive control</li> <li>• Sample assigned incorrectly</li> </ul>	<ul style="list-style-type: none"> <li>• Repeat run and investigate the source of contamination (Section 7).</li> <li>• Ensure sample is assigned accordingly</li> </ul>
<b>Test sample</b>	Colour changed from pink to orange	<ul style="list-style-type: none"> <li>• Very low copy sample</li> </ul>	<ul style="list-style-type: none"> <li>• return reactions to 63 °C for additional 15 mins.</li> <li>• place reaction vessels on ice for 15-30 seconds. This will intensify the color.</li> </ul>

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**10. Limitations**

- The use of this product and its data interpretation is intended for personnel trained in laboratory and *in vitro* diagnostics procedures only.
- Appropriate sample collection, transport, storage, and nucleic acid extraction procedures are required for reliable results.

**11. Explanation of Symbols**

Symbol	Explanation
	Research use only
	Catalogue number
	Store at -25°C to -15°C
	Manufacturer
	Lot number
	Use by
	Contains sufficient for <n> tests
	Important

**12. References**

1. (2020) Coronavirus disease (COVID-19) Pandemic. [Online] WHO. [Accessed 20 April 2020].
2. (2020) 2019 Novel Coronavirus (2019-nCoV), Wuhan, China. [Online] US Centers for Disease Control and Prevention. [Accessed 27 Jan 2020].
3. Zhu N, Zhang D, Wang W, Li X, Yang B et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med.* 2020; 1-7.
4. (2020) Naming the coronavirus disease (COVID-19) and the virus that causes it [Online] WHO. [Accessed 8 May 2020].

Electronic copy of the product insert can be downloaded at <http://aitbiotech.com/covid-19/>