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Technical Information

Isopollo® COVID-19 detection kit (real-time)

Overview

Isopollo® COVID-19 detection kit (real-time) is designed for the detection of novel coronavirus (COVID-19) infection from extracted RNA of specimens by RT-LAMP (Reverse transcription loop-mediated isothermal amplification) and it has high specificity for targeted RNA because 6 primers selectively detect specific RdRP gene, N gene of coronavirus.

Intended Use

Isopollo® COVID-19 detection kit (real-time) is for qualitative analysis to detect novel coronavirus (COVID-19) infection from extracted RNA of specimens by RT-LAMP (Reverse transcription loop-mediated isothermal amplification) used together with real-time PCR Instrument, CFX96™ Dx System (Bio-Rad, Model. 12007917) or equivalent. This device should be used by professional.

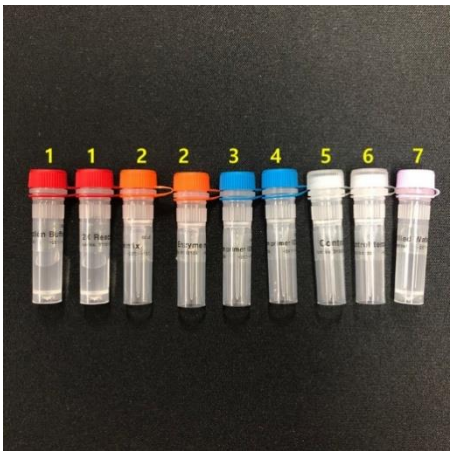
Principles of the examination method

LAMP (Loop-mediated isothermal amplification) is a new concept of high-efficiency amplification that can be performed synapsis and protraction at a constant temperature using *Bst* polymerase with strand displacement DNA synthesis. *Bst* polymerase can be performed synapsis and protraction directly at synapsis temperature which is close to the Tm value without thermal denaturation of DNA double helix structure, because *Bst* polymerase has 5 '→ 3' exonuclease function unlike *Taq* polymerase that is commonly used in general PCR. The amplification reaction is initiated by six primers. First, inner primer binds to the DNA and then extends. Then, the outer primer binds and extends to the outer primer and strand displacement occurs. When strand displacement is generated, the first strand is formed off.

The loop structure is formed from the 5'-end of the single strand that is separated from the loop. This same process repeats at the 3'-end and extends the loop structure. In order to perform the LAMP, specifically designed six primers are used to recognize the six positions of the genes to be amplified. It means that the specificity of the target sequence is very high compared to typical PCR that recognizes the two positions. Therefore, LAMP method can check gene amplification by graph using fluorescence, the results can be checked quickly in real-time without electrophoresis or other processes.

The testing procedure of the product is performed with Three (3) steps of: 1) RNA extraction from specimens; 2) Target gene amplification and; 3) detection by the fluorescence.

Components



No.	Component	Number of tube	Volume (ul/tube)	Appearance
1	2X Reaction Buffer	2 EA	625 ul	Colorless transparent liquid in clear plastic tube with red cap
2	Enzyme mix	2 EA	50 ul	Colorless transparent liquid in clear plastic tube with orange cap
3	Detection primer (CR)	1 EA	100 ul	Colorless transparent liquid in clear plastic tube with blue cap
4	Detection primer (CN)	1 EA	100 ul	Colorless transparent liquid in clear plastic tube with blue cap
5	Control primer	1 EA	20 ul	Colorless transparent liquid in clear plastic tube with transparent cap
6	Control template	1 EA	20 ul	Colorless transparent liquid in clear plastic tube with transparent cap
7	Distilled water	1 EA	1.0 ml	Colorless transparent liquid in clear plastic tube with violet cap

Storage and shelf life after first opening

- Isopollo® COVID-19 detection kit (real-time) is shipped on ice pack. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact BioActs Inc., for assistance.
- All components should be stored between -25°C and -15°C upon arrival.
- Repeated thawing and freezing of reagents should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Storage between +2°C and +8°C should not exceed a period of two hours.

Shelf life	12 months	-25 to -15°C

Warnings and Precautions

1) When handling the samples, always comply with the rules for biohazard regulation to prevent infections from unknown microbes or diseases. After finishing experiment, dispose the laboratory wastes considering as biological wastes.

2) This kit is very sensitive. Thus, it can be easily contaminated by own amplified products. If conducting electrophoresis (usually not necessary for our protocol), highly cautious attention is needed especially when opening the cap of final reaction tubes.

3) This kit should be stored at -25℃ ~ -15℃. We recommend taking out the necessary amount of reagents from the freezer before use to prevent deterioration of the reagents. Do not repeat unnecessary freezing and thawing. When thawing the reagents, remain them at room temperature a while, after thawing keep them on ice for preparation step. If storing for a long time, keep the storage temperature much lower as -80℃.

4) Before the reaction, mix the solution in PCR tubes well and then spin down the tubes to drop down the solution staying on the tube wall or on the cap. Notice that fierce mixing should be avoided as it can inactivate the enzyme.

5) Since bubbles in the solution will interfere the exact judgment, try not to cause any bubble when mixing the solution. If bubbles are present, spin down to get rid of the bubbles.

6) Keep the cap of the used tube completely closed and dispose it, according to the relevant regulations and instructions, by incineration or after double bagging it with sealable vinyl bag.

7) This kit is designed for research use only.

Additional required equipment

The Isopollo® COVID-19 detection kit (real-time) was developed and validated to be used with the following real-time PCR instruments:

real-time PCR instrument	Device name	CFX96™ Dx System
	Catalog Number	12007917
	Manufacturer	Bio-Rad, USA
Other devices required	• Appropriate nucleic acid extraction system or kit • Desktop centrifuge with a rotor for 2 ml reaction tubes • Vortex mixer • Pipettes (adjustable) • Pipette tips with filters (disposable) • Sterilized tubes (0.2 mL real-time PCR tube, 1.5 mL tube) • Aluminum rack for cooling or ice box • Centrifuge • Heat block or real-time PCR machine • Protective goggle or glass board, and powder-free gloves (disposable)	

i	Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.

Protocol

1. Sample preparation and nucleic acid extraction

① The collected samples should be used immediately or stored at -20℃.

② Nucleic acid should be isolated using useful viral RNA extraction kit according to the manufacturer's instructions.

2. Reagents preparation & Reaction

① Take out the Lamp premix (tube) stored at -20℃, and thaw them at room temperature. Once the reagents are thawed, keep them on ice.

② Prepare 25㎕ LAMP reaction mixture as follows:

- Check for RdRP gene

Reagents	Volume (1 Reactions)
2X Reaction Buffer	12.5 ㎕
Enzyme mix	1.0 ㎕
Detection primer (CR)	2.0 ㎕
Extracted RNA (Template)	2.0 ~ 9.5 ㎕
Distilled water*	- ㎕
Total	12.5 ㎕

☞ If the number of samples to be examined is so many, it is recommended that the mixture be calculated according to the number of responses and used in a 1.5 ml tube.

☞ In case of distant water, adjust and add according to the template volume.

☞ The positive/negative control is made and used if necessary. The positive control adds 2㎕ of the control template, 2㎕ of the control primer, 7.5㎕ of the distant water, and in the case of the negative control group, the distilled water adds instead of the control template.

- Check for N gene

Reagents	Volume (1 Reactions)
2X Reaction Buffer	12.5 ㎕
Enzyme mix	1.0 ㎕
Detection primer (CN)	2.0 ㎕
Extracted RNA (Template)	2.0 ~ 9.5 ㎕
Distilled water*	- ㎕
Total	12.5 ㎕

☞ If the number of samples to be examined is so many, it is recommended that the mixture be calculated according to the number of responses and used in a 1.5 ml tube.

☞ In case of distant water, adjust and add according to the template volume.

☞ The positive/negative control is made and used if necessary. The positive control adds 2㎕ of the control template, 2㎕ of the control primer, 7.5㎕ of the distant water, and in the case of the negative control group, the distilled water adds instead of the control template.

3. Operation procedure

① Select wavelength of FAM or SYBR Green 1 in real-time PCR machine(CFX96 Dx system, Bio-Rad, CA, USA) and perform the reaction as follows:

Step	Temperature	Time
1	58 °C	30 min
2	80 °C	2 min

4. Detection

- Positive criterion Ct value : within 40 Ct

Quality Control

This product contains positive control in the product. Users should react the positive and negative control individually and observe if they work normally to determine the performance. Users may request the replacement if abnormal result are obtained within appropriate storage environments and validity period.

Interpretation of Results

After the LAMP reaction is completed, remove the tube from the device and compare the color to determine the result. The result is positive If Ct value is within 40 Ct, negative if Ct value is not detected.

	Criterion Ct value
Positive	Within 40 Ct
Negative	Not detection

Performance characteristics

1. Analytical Performance

1) Analytical Sensitivity

Based on CLSI EP17, tests were repeated 30 times per each reference material. The limit of detection was measured 1×10^3 copies/㎕ for RdRP gene and 1×10^3 copies/㎕ for N gene.

Virus	Gene	LoD
Novel coronavirus COVID-19	COVID-19 RdRP	$1X10^3$ copies/㎕
	COVID-19 N	$1X10^3$ copies/㎕

Example)

- COVID-19 RdRP (CR)

Lane	**1**	**2**	**3**	**4**	**5**	**6**	**7**
(copies/㎕)	$1X10^5$	$1X10^4$	$1X10^3$	$1X10^2$	$1X10^1$	1	Negative Control
Ct value	12.83	14.75	19.26	N/A	N/A	N/A	N/A

- COVID-19 N (CN)

Lane	**1**	**2**	**3**	**4**	**5**	**6**	**7**
(copies/㎕)	$1X10^5$	$1X10^4$	$1X10^3$	$1X10^2$	$1X10^1$	1	Negative Control
Ct value	12.83	16.92	19.23	N/A	N/A	N/A	N/A

2) Analytical Specificity

12 test microorganisms listed in the KFDA Guideline, "Influenza virus and hepatitis A virus guideline" were tested for cross-reactivity. None of the 12 species of test microorganism demonstrated cross reactivity.

No.	**Test microorganisms**	**Cross-reactivity**
1	*Measles*	None
2	*Cytomegalovirus*	
3	*Eastern Equine Encephalitis Virus*	
4	*Hepatitis A virus*	
5	*Herpes virus (HSV)*	
6	*Borrelia Burgdorferi*	
7	*Saint Louis Encephalitis Virus*	
8	*Respiratory Syncytial Virus B*	
9	*Escherichia coli*	
10	*Pseudomonas Aeruginosa*	
11	*Influenza A*	
12	*Parainfluenza virus 1*	

Example)

- COVID-19 RdRP (CR)

* Lane	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**PC**
Ct value	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	13.16

- COVID-19 N (CN)

*** Lane**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**PC**
Ct value	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	19.41

* Lane: Lane information is equal to above test microorganisms.

The summary of interference test result is presented in the table below. Interference was observed by blood at the highest interfering concentration suggested in KFDA Guideline, "Influenza virus and hepatitis A virus guideline" and CLSI EP07-A. However, this kind of interference is not the case of consideration, since purified nucleic acids is used for the amplification reaction by Isopollo® COVID-19 detection kit (real-time).

No.	Interfering substance	Interfering concentration
1	Mucin	No detection
2	Blood	≥ 5g/dL
3	Mupirocin	No detection
4	Tobramycin	No detection
5	Zanamivir	No detection

3) Precision

Reproducibility test is composed of sets of tests using three different Plasmid DNA concentrations of three lots of products. Each test set was performed in triplicate and 2 runs of test set was done per day, morning and afternoon, for 5 days by each assigned operator at three sites.

- The result of reproducibility about COVID-19 RdRP (CR)

Concentration	Replicates	Concordance (%)					
		Inter-site	Inter-operator	Intra-assay	Inter-assay	Inter-day	Inter-lot
High positive	90	100	100	100	100	100	100
Mid positive	90	100	100	100	100	100	100
Low positive	90	100	100	100	100	100	100
Negative control	90	100					

- The result of reproducibility about COVID-19 N (CN)

Concentration	Replicates	Concordance (%)					
		Inter-site	Inter-operator	Intra-assay	Inter-assay	Inter-day	Inter-lot
High positive	90	100	100	100	100	100	100
Mid positive	90	100	100	100	100	100	100
Low positive	90	100	100	100	100	100	100
Negative control	90	100					

Repeatability test was performed 2 sets per day, morning and afternoon, for 5 days. Each set includes triplicates of tests using three different Plasmid DNA concentration such as standard material and three lots of products. The precision of intra-assay, inter-assay, inter-lot, and inter-day tests demonstrated 100% concordance.

- The result of repeatability about COVID-19 RdRP (CR)

Concentration	Replicates	Concordance (%)			
		Intra-assay	Inter-assay	Inter-lot	Inter-day
High positive	30	100	100	100	100
Mid positive	30	100	100	100	100
Low positive	30	100	100	100	100
Negative control	30	100			

- The result of repeatability COVID-19 N (CN)

Concentration	Replicates	Concordance (%)			
		Intra-assay	Inter-assay	Inter-lot	Inter-day
High positive	30	100	100	100	100
Mid positive	30	100	100	100	100
Low positive	30	100	100	100	100
Negative control	30	100			

Custom Labeling Service

Based on accumulated know-how and technologies, BioActs provide a wide range of custom services such as protein fluorescence labeling, organic synthesis, oligonucleotide synthesis upon customers' request. Our reliable technology has acknowledged by our clients from domestic and overseas universities, institutions, in vitro diagnostic and pharmaceutical companies and has enabled to steadily conduct their requirements. In addition, we can introduce fluorescent materials to many other compounds such as organic and inorganic compounds, drugs, hormones, polymer, peptides, proteins, antibodies, etc. We also can provide chemical and optical analytical data, along with cell and animal experiments.

Technical Support

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SDS (Safety Data Sheets) You can find SDS at www.bioacts.com, the official website of BioActs.
CoA (Certificate of Analysis) provides detailed quality information of each product. To see CoA, check the lot number written on each product's page at www.bioacts.com, when having trouble with check, contact to our technical support team

WARNING: This product can be used for research use only (RUO) or primary screening purpose to other regions except for South Korea.
This product is intended for research or screening for COVID-19 virus detection.
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