BioActs Co., Ltd.
9, Cheongneung-daero 595beon-gil, Namdong-gu, Incheon 21666, Korea
Tel: +82-32-818-9100, Fax: +82-32-818-8206
www.bioacts.com • technical@bioacts.com

Technical Information

Isopollo® COVID-19 detection kit (premix)

Overview

Isopollo® COVID-19 detection kit (premix) 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. After completion of the reaction, the results can be confirmed immediately by color change.

Intended Use

Isopollo® COVID-19 detection kit (premix) 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 Isothermal Amplification Instrument or PCR Instrument, SimpliAmp Thermo Cycler (Thermo Fisher, Model. A24811) or equivalent. This device should be used by professional.

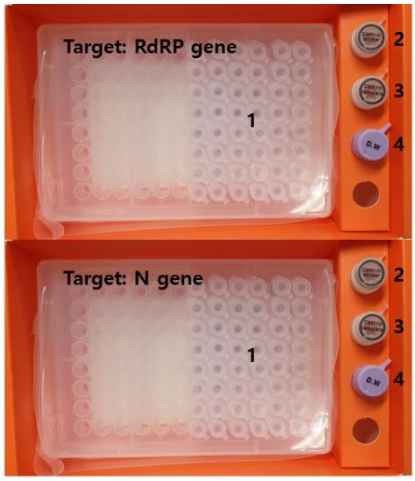
Principles of the examination method

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. Amplification reaction at a constant temperature can be shortened the reaction time. Also, additional processes in the conventional PCR, such as electrophoresis can be reduced by checking the results visually using the coloring dye. Several papers have introduced it as onsite technique to obtain rapid results without any expensive equipment while maintaining high sensitivity and specificity compared to conventional molecular detection methods.

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 color change from violet to sky blue.

Components



Target	No.	Component	Number of tube	Volume (ul/tube)	Appearance
RdRP gene	1	Lamp premix (CR)	48 EA	15 ul	Purple transparent liquid in clear plastic tube with transparent cap
	2	Control primer	1 EA	20 ul	Colorless transparent liquid in clear plastic tube with transparent cap
	3	Control template	1 EA	20 ul	Colorless transparent liquid in clear plastic tube with transparent cap
	4	Distilled water	1 EA	1.0 ml	Colorless transparent liquid in clear plastic tube with violet cap
Target	**No**	**Component**	**Number of tube**	**Volume (ul/tube)**	**Appearance**
N gene	1	Lamp premix (CN)	48 EA	15 ul	Purple transparent liquid in clear plastic tube with transparent cap
	2	Control primer	1 EA	20 ul	Colorless transparent liquid in clear plastic tube with transparent cap
	3	Control template	1 EA	20 ul	Colorless transparent liquid in clear plastic tube with transparent cap
	4	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 (premix) 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	6 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) Do not expose the Lamp premix (tube) to UV light and dry ice. A change in color or degeneration caused by ultraviolet lamp and dry ice sometimes results in misjudgment.

8) This kit is designed for research use only.

Additional required equipment

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

PCR instrument	Device name	SimpliAmp™ Thermal Cycler
	Catalog Number	A24811
	Publication Number	MAN0009889
	Manufacturer	Thermo Fisher Scientific, 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 PCR tube, 1.5 mL tube) • Aluminum rack for cooling or ice box • Centrifuge • Heat block or 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 Ten microliter (10㎕) of extracted nucleic acid is put in Lamp premix tube. After closing caps, they are thoroughly mixed by tapping the tubes 2~3 times, and then spin down.

☞ For control reactions, use 2 μL of Control template and 2 μL of Control primer as positive control and use DW instead of RNA as negative control.

3. Operation procedure

① Incubate at 58℃ for 30minutes and inactivate the reaction by heating at 80℃ for 2 minutes as follows:

Step	Temperature	Time
1	58 ℃	30 min
2	80 ℃	2 min

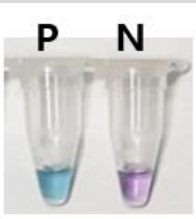
4. Detection

① Visual Colorimetric detection

- Negative result shows purple color, while positive shows sky blue.

Color code	Color	Judgment
	Sky blue	Positive
	Purple	Negative

(Example)

Examples of reactions	Readings
	P : Positive (Sky blue) N : Negative (Purple)

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 color is sky blue, negative if color is purple.

	Color	Color chart	Color code (HEX)
Positive	Sky blue		#416B97
Negative	Purple		#744F8F

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 1×10^4 copies/㎕ for RdRP gene and 1×10^3 copies/㎕ for N gene.

Virus	Gene	LoD
Novel coronavirus COVID-19	COVID-19 RdRP	$1X10^4$ copies/㎕
	COVID-19 N	$1X10^3$ copies/㎕

Example)

- COVID-19 RdRP (CR)

Lane (copies/㎕)	1	2	3	4	5	6	7
	$1X10^5$	$1X10^4$	$1X10^3$	$1X10^2$	$1X10^1$	1	Negative Control
Result	+	+	-	-	-	-	-

- COVID-19 N (CN)

Lane (copies/㎕)	1	2	3	4	5	6	7
	$1X10^5$	$1X10^4$	$1X10^3$	$1X10^2$	$1X10^1$	1	Negative Control
Result	+	+	+	-	-	-	-

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 (copies/㎕)	1	2	3	4	5	6	7	8	9	10	11	12	PC
Result	-	-	-	-	-	-	-	-	-	-	-	-	+

- COVID-19 N (CN)

Lane (copies/㎕)	1	2	3	4	5	6	7	8	9	10	11	12	PC
Result	-	-	-	-	-	-	-	-	-	-	-	-	+

* 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 (premix).

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

ADDRESS
BioActs CO., LTD. DK Tower 10TH F., 595 beon-gil 9, Cheongneung-daero, Namdong-gu, Incheon, 21666, Korea

PHONE & FAX
Tel: +82-32-818-9100
Fax: +82-32-818-8206

WEBSITE
http://www.bioacts.com/

MAILS
order@bioacts.com (Order Support)
support@bioacts.com (Customer Support)
ivd@bioacts.com (B2B/Bulk Order Support)

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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