BIOPOA

POA- nCoVn- LFD- 16 | **Speed COVID- 19 LFD kit** | **16 rxn**

Revision No.: POA-nCoV_LFD-Orf1a-001
Issue Date: Dec. 20. 2020
User Manual

•Distributed by BioPOA Co., Ltd.
•Web: www.biopoa.co.kr Tel: +82-31-375-8304 Fax:+82-31-375-8305
•593-26, Dongtangiheung-ro, Hwaseeong-si, Gyeonggi-do, 18469, Republic of Korea

•Manufactured by: COSMAX PHARMA CO., Ltd
•168-23, Osongsaengmyeong 4-ro, Osong-eup, Heungdeok-gu, Cheongju-si, Chungcheongbuk-do, Republic of Korea

1. General Information

The isothermal TwistAmp technology is based on the Recombinase Polymerase Amplification (RPA) process developed by TwistDx Ltd. The amplification products generated by RPA can be detected by lateral flow test strip, PCRD Nucleic Acid Detector supplied by Abingdon Health Ltd.

2. Application

This Speed COVID-19 LFD kit contains specially designed probe and primer set for amplification of Orf1a gene to detect COVID-19 (2019-nCoV) from clinical samples.

3. Product Description

Novel coronavirus, also known as 2019-nCoV Wuhan coronavirus and Wuhan seafood market pneumonia virus is a positive-sense, single-stranded RNA coronavirus. The first suspected cases were notified to the WHO on 31 December 2019, with the first instances of symptomatic illness appearing just over three weeks earlier on 8 December 2019. The virus was genomically sequenced from a sample from a person with pneumonia during the 2019–20 Wuhan coronavirus outbreak.

Reported symptoms have included fever, fatigue, dry cough, shortness of breath, respiratory distress, pneumonia, kidney failure and death in severe cases. Among the majority of those hospitalized, vital signs were stable on admission, and they had leukopenia and lymphopenia. However, a quarter among those infected have experienced severe symptoms. Most of these patients also presented underlying conditions such as hypertension, diabetes or cardiovascular disease.

This virus belongs to the family of coronaviruses. Coronaviruses form a large family of viruses, and the illnesses they cause can range from the common cold to more severe diseases such as the Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS). Coronaviruses are a broad family of viruses, but only six (229E, NL63, OC43, HKU1, MERS-CoV, and SARS-CoV) were previously known to infect people; 2019-nCoV made it seven.

The Speed COVID-19 LFD kit contains three parts of a specific ready-to-use system for the detection of nCoV, RNA extraction, Gene amplification, and Amplicon detection on site manner. RNA extraction is supported by Arcis Biotechnology Ltd within a few minutes. Gene amplification is supported by newly developed isothermal gene amplification technology, called RPA method (TwistDx Ltd). The master contains enzymes and reagents including specific primer set which is specially designed to amplify the Orf1a gene for the unique amplification of nCoV within 20 min at room temperature. The labelled amplicon can be detected in a few minutes by PCRD Nucleic Acid Detector which is a single-use *in vitro* immunochromatographic test supported by Abingdon Health Ltd. Therefore, total reaction can be done within 30 minute without expensive equipment.

4. Storage condition

1) The kit should be keep at -20℃.
2) Do not freeze and thaw the kit frequently.

5. Specimen storage condition

1) The specimen can be kept at 4℃ up to 72 hours. For keeping it for more time than 72 hours, it is should be kept it under -70℃.
2) Viral RNA extraction kits are available from various manufactures. You can use your own extraction systems or commercial kits.
3) Extracted RNA should be keep under -70℃.

6. Kit contents

No		Name	Q'ty / 16 rxn	Storage condition
Rapid RNA Prep Kit	1	Lysis Buffer (White tube)	150 ㎕, 16 ea	4℃
	2	Wash Buffer (Orange tube)	20 ㎕, 16 ea	4℃
COVID-19 LFD Kit	3	COVID-19 LFD Primer Mix	647 ㎕, 1 ea	-20℃
	4	COVID-19 probe	13 ㎕, 1 ea	-20℃
	5	LFD Enzyme mix	Lyophilized tube, 16 ea	-20℃
	6	280 mM magnesium acetate	50 ㎕, 1 ea	-20℃
	7	Positive control (Orf1 gene-DNA)	10 ㎕, 1 ea	-20℃
	8	Molecular grade Distilled water	500 ul, 1 ea	-20℃
PCRD Detector	9	Extraction Buffer	5 ml, 3 ea	*RT
	10	PCRD Nucleic Acid Detector LFD	16 ea	RT

7. Required Materials and Devices

Micropipets (10 – 200 ㎕) / Sterile filter tips (10, 20, 200 ㎕) / Refrigerator / Freezer / Tube racks / Biohazard waste container

8. RNA extraction

* Corona virus RNA can be extracted easily in a few minutes with Arcis DNA Sample Prep kit made by Arcis Biotechnology Ltd.
1) Put less than 90 ㎕ of clinical samples from sputum, nasopharyngeal swab, oropharyngeal swab, stool, urine etc into white tube (lysis buffer) of Rapid RNA prep kit.
2) Mix it well and incubate 1 minute at room temperature.
3) Transfer 5 ㎕ of it into orange tube (wash buffer).
4) Use 5 ㎕ in RPA reaction in next step.

9. RPA reaction

1) Prepare the reaction mixture as shown in Table 2.
2) Transfer 48 ㎕ into LFD Enzyme mix and mix well.
3) Transfer 2 ㎕ of RNA extract into it.
4) Mix it well and incubate 20 min at 37 ℃.

No	Contents	Volume		
		1 reaction	8 reaction	16 reaction
1	COVID-19 LFD Primer Mix	34.7 ㎕	277.6 ㎕	555.2 ㎕
2	COVID-19 probe	0.6 ㎕	4.8 ㎕	9.6 ㎕
3	280 mM magnesium acetate	2.5 ㎕	20.0 ㎕	40.0 ㎕
4	Molecular grade Distilled water	10.2 ㎕	81.6 ㎕	163.2 ㎕
Total		48.0 ㎕	384.0 ㎕	768.0 ㎕

10. Amplicon Detection

1) Prepare the PCRD Nucleic Acid Detector.
2) Mount 20 ㎕ of RPA reaction on the rim of device.
3) Add 90 ㎕ of PCRD extraction buffer for dropping down into the sample hole of PCRD device.
4) Leave the PCRD device to develop for 10 minutes while the result develops.
5) Read the results visually at 10 minutes. Ignore any changes which occur after 10 minutes.

11. Performance Characteristics

1) Assay range: For guidance, in lab studies PCRD device has been shown to detect between 0.02 ug/ml and 0.001 ug/ml ds DNA. However, levels of sensitivity will depend on the outcome of your own RPA reaction. The detection level for your assay should therefore be determined empirically.

2) Specificity: PCRD device is specific for the detection of DIG and FITC. It does not detect Dinitophenol (DNP) of Sulforhodamine 101 Acid Chloride (Texas Red).

12. Considerations

1) An initial quality control check on your primer pair and DNA is advised. PCRD device is highly sensitive and may detect the presence of primer dimers and non-specific product as a "positive".

2) Keep the LFD in protective container for as long as possible to reduce the risk of cross contamination.

13. Reading and Result

<Example of RT-RPA LFD reaction with Positive and Negative control (Distilled water)>

1) The Positive reaction shows dark band in line 2 and line C (control line)
2) The Negative control and distilled water show only one band in line C.
3) If the negative control or distilled water show the positive reaction, please repeat again the experiment.
4) If the false positive reaction shows again even in the repeated experiments, clean the experimental environment with RNase away or 70% alcohol including pipets, also. And use new cotton pipet tips.

14. Warnings and Precaution

1) Carefully read this instruction before starting the procedure.
2) Clinical samples should be regarded as potentially infectious materials and prepared the RPA reaction mixture in a clean area.
3) Do not use the kit after its expiration date written on box.
4) Avoid repeated thawing and freezing of the reagents, this may cause wrong test result.
5) Once the reagents have been thawed, vortex, and spin down briefly the tubes before use.
6) Prepare quickly the reaction mixture on ice.
7) Use always sterile pipette tips with filters.
8) Wear separate coats and gloves in each area.
9) Collected test samples in sterile tubes.
10) Test samples should extracted immediately or frozen at -20℃ to -80℃.