Novel Coronavirus (2019-nCoV) Real-time Isothermal Amplification Kit

Instruction for Use

Label	Description
	Contains sufficient for <N> tests
	Do not re-use
	Batch number
	Date of manufacturing
	Date by which the device should be used
	Name and address of Manufacturer
	In vitro diagnostic
	Consult instruction before use
	Temperature limitation

March 2020 — Version 1.1

【PRODUCT NAME】

Novel Coronavirus (2019-nCoV) Real-time Isothermal Amplification Kit (Commercial names: Fosun 2019-nCoV rapid)

【SIZE】

32 tests/kit, 48 tests/kit, 96 tests/kit, 1test*32/kit

【INTENDED USE】

This product is intended for the rapid detection of 2019-nCOV in human nasopharyngeal swab, throat swab or sputum samples.

This product is helpful for clinical diagnosis of 2019-nCOV infection. The test results are only for clinical reference, and can't be used as the basis for diagnosis or exclusion of cases. The experimental operators should have received the professional training of gene amplification or molecular biological method detection, have the relevant experimental operation qualification, and the laboratory should have the corresponding biological safety protection conditions.

The common signs of people infected with coronavirus are respiratory symptoms, fever, cough, shortness of breath and dyspnea. In more serious cases, infection can lead to pneumonia, severe acute respiratory syndrome, renal failure, and even death. The novel 2019 coronavirus was discovered in 2019 in Wuhan, and was named as "2019-nCoV" by WHO in January 12, 2020. It confirmed that it can cause colds and more serious diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS).

【PRINCIPLE OF DETECTION】

Novel coronavirus (2019-nCoV) specific conserved sequence was detected by isothermal amplification method in this kit. The principle is to reverse transcribe the coronavirus RNA into cDNA by reverse transcriptase, and then use the Bst DNA polymerase with chain replacement function to amplify specifically with cDNA as the template at a constant temperature. The amplification process can be divided into two stages: the start-up stage and the cycle amplification stage, and finally formed double stranded DNA mixture. The fluorescence signal was generated when the amplification product combines with nucleic acid dye, which can be captured by the instrument, processed and analyzed to form the amplification curve, and judge the detection result according to the Tt value.

【PRODUCT CONTENTS】

Components	Amount				Amount per reaction	Ingredient
	32 Tests/kit	48 Tests/kit	96 Tests/kit	1test*32/kit		
2019-nCoVReaction Reagent	576μL	864μL	864μL*2	18μL*32 Tubes	18μL	dNTPs, $MgCl_2$, Primers
Mix Enzyme	64μL	96μL	96μL*2	2μL*32 Tubes	2μL	Bst FL polymerase, RT II
Positive Control of 2019-nCoV	200μL	200μL	200μL	200μL	-	Nucleic acid template
Negative Control	200μL	200μL	200μL	200μL	-	NaCl

Note: 1. Do not mix the components from different batches for detection. The positive control of 2019-nCOV was constructed artificially, and they were not infectious.

2. Self-provided experimental consumables: swab sampling tube, etc.

【STORAGE & SHELF LIFE】

All reagents should be stored at -15°C~-25°C with protection from light, and the reagents are stable for 6 months (to be determined) when stored at the recommended condition. See label for production date and expiration date.

The kit should be transported by cold chain transport or sealed foam box with ice. The temperature should be controlled below -8°C and the transportation time should not exceed 4 days. Repeated freeze-thaw should be less than 5 times.

【INSTRUMENTS】

Recommendation of platform Isothermal: Nucleic acid Amplification Analyzer --Vela-1000/32, Real-time PCR instrument Applied Biosystems series and SLAN series.

【SAMPLING & HANDING】

(1) Nasopharynx swab: Press the nasopharynx swab against the nasal septum and slowly penetrate into the back of nasopharynx, rotate it several times to obtain secretion, and quickly immerse the swab into the sample collection tube, discard the tail, and tighten the tube cover to seal to prevent drying.

(2) Throat Swab: Use the plastic rod swab with polypropylene fiber head to wipe the bilateral pharyngeal tonsils and the posterior pharyngeal wall at the same time, immerse the swab head into the tube containing physiological saline, discard the tail, and tighten the tube cover.

(3) Sputum: Cough up the sputum in the deep part of the respiratory tract and collect it in the container. Liquefying method: add equal volume of acetylcysteine (10g/L) into the sputum sample, shake at room temperature for 30 minutes, and then carry out RNA extraction after sufficient liquefying.

The sample can be stored for 24 hours at room temperature, 4 daysat 2~8℃, and for long time below -20℃.

Samples shall be transported at low temperature in accordance with biosafety regulations.

【PROTOCOL】

1. Reagent Preparation

Prepare reaction reagent according to the number of reaction samples (number of reaction samples, n = number of samples to be tested + 2 control samples):

Add n × 2μL of mix enzyme and n × 18μL of Cov reaction reagent into the centrifuge tube, mix by shaking, and centrifugate at low speed for a few seconds, then make aliquots of 20μL into different PCR reaction tubes. The reaction tubes can be placed at 2~8℃ for 3 hours after separation.

2. Nucleic acid Extraction

It is recommended to use the QIAamp Viral RNA Mini Kit (Qiagen) and NX-48 Viral RNA Kit (Genolution) to extract nucleic acid from sample and reference sample.

Take 200μL of sample to be tested (quantity n) and control sample (quantity 2) for nucleic acid extraction. After extraction, the extracted nucleic acid shall be added to the reaction tubes within 10 minutes, or transferred to the centrifuge tubes and stored at -15 ℃~-25 ℃.

3. Template Addition

Add 5μL of extracted Negative Control, 5μL of extracted Positive Control, and 5μL of extracted nucleic acid from sample to different PCR reaction tubes. Centrifuge them at low speed. Then, move them to the Constant temperature PCR instrument.

4. Constant temperature Amplification

63℃ for 30 minutes. The signal of SYBR or FAM channel was collected by every 30 seconds.

5. Data Analysis

The results are determined according to the threshold time (Tt value) of the instrument test.

6. Quality Control

Negative control and positive control provide the calibration for the kit, and shall be set for each test. The result is valid if ALL the below criteria is met. Otherwise, the test is invalid. In this case, the errors of instruments, reagents, amplification conditions, etc. shall be checked, and the experiment shall be repeated.

Products of Quality Control	Requirements of Quality Control
Negative Control	Undet
CoV Positive Control	Amplification curve is typical S shape, and Tt ≤ 30

【CUT-OFF VALUE OR REFERENCE INTERVAL】

The cut-off value of this kit is 30. When the test sample's Tt value ≤ 30, it is determined to be positive. When there is no Tt value, it is determined to be negative.

Positive amplification curve is typical S shape, and the fluorescence signal of amplification end should be significantly larger than the start-up.

【ASSAY EXPLAINATION】

1. The contamination of laboratory environment and reagent, or cross contamination during specimen treatment may lead to false positive result.

2. The decrease of detection effect even the false negative result may occur if there is any mistakes in the transportation, storage and operation of reagents.

【ASSAY LIMITATIONS】

1. The positive result detected by this kit can't indicate whether there is virus in vivo. It is suggested to use other methods for confirmation at the same time.

2. This kit is intended for detection of 2019-nCOV. The result is only for clinical reference, and the clinical management of patients should be considered in combination with their symptoms/signs, history, other laboratory tests and treatment responses.

3. Although the detected target sequences of this kit are the conservative region of gene, the missed detection of coronavirus types with rare mutations in the conservative region can't be completely avoided in theory.

【PERFORMANCE SPECIFICATIONS】

1. Detection limitation: 500copies/mL.

2. Conformity rate of Positive Control: detect the enterprise reference positive samples (Y1-Y5), the detection result was 100% (5/5).

3. Conformity rate of Negative Control: detect the enterprise reference negative samples (T1-T15), the detection result was 100% (15/15).

4. Precision: The test results of enterprise reference samples (J1-J2) were all positive after 10 repetitions.

5. Specificity: non-specific interference of other related pathogens. Normal concentrations of hemoglobin, albumin, mucus, antifungal drugs and other interfering substances were added to the simulated samples, which did not interfere

with the test results.

【ATTENTIONS】

1. The kit is only used for *in vitro* diagnosis.
2. Please read this manual carefully before beginning the experiment.
3. All equipment used in the experiment shall be sterilized.
4. Unreasonable sample collection, transfer, storage and operation may lead to wrong test results.
5. Extraction of nucleic acid shall be carried out as soon as possible after sample collection to avoid degradation. If it cannot be carried out immediately, it shall be stored in accordance with **[SAMPLING & HANDING]**.
6. As this test involves the extraction and amplification of pathogen, please take care to avoid contamination of the amplification reaction mixture. Regular monitoring of laboratory contamination is recommended.
7. The clinical laboratory should be equipped with equipment and operators in strict accordance with the *working standard of clinical gene amplification laboratory*.
8. When using this kit, please strictly follow the instructions. The processes of sample preparation and addition must be carried out in the biosafety cabinet or other basic protective facilities according to the technical requirements of the clinical gene amplification laboratory.

【REFERENCE】

[1] Surveillance of novel coronavirus infection cases (2nd ed).

[2]Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected.WHO.2020.

【General Information】

Manufacturer:Shanghai Fosun Long March Medical Science Co., Ltd

Production license No.: Medical device production license No. 20020886 by Shanghai Food and Drug Administration