AmoyDx® Novel Coronavirus (2019-nCoV) Detection Kit

Detection of novel coronavirus SARS-CoV-2

Instruction for Use

For Medical Professional Use Only

REF 8.0131901X096E 96 tests For Stratagene Mx3000P™, ABI 7500, LightCycler 480 II, SLAN-96S

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Background

The novel coronaviruses belong to the β genus. COVID-19 is an acute respiratory infectious disease. People are generally susceptible. Currently, the patients infected by the novel coronavirus are the main source of infection; asymptomatic infected people can also be an infectious source. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. The main manifestations include fever, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.

Intended Use

The AmoyDx® Novel Coronavirus (2019-nCoV) Detection Kit is a real-time reverse transcription (RT)-PCR assay for qualitative detection of novel coronavirus SARS-CoV-2. The kit is intended use with viral RNA extracted from human upper and lower respiratory specimens. The kit is intended to be used by trained professionals in a laboratory environment.

Principles of the Procedure

The kit is based on real-time RT-PCR technology, combines reverse transcription and PCR amplification in one-step procedure. It's designed for a specific amplification of the ORF1ab (open reading frame, ORF1ab) and N (Nucleoprotein, N) conserved regions of novel coronavirus SARS-CoV-2 in viral RNA. The targeted region is amplified by several specific primers and detected by fluorescence probes. A non-competitive internal control is included in nCoV RNA detection system to assess RNA quality and monitor the whole PCR procedure.

The kit is composed of **nCoV Reaction Mix, nCoV Enzyme Mix** and **nCoV Positive Control**.

1) The **nCoV Reaction Mix** includes nCoV RNA detection system and an internal control system. It contains primers and FAM-labeled probe specific for ORF1ab gene of SARS-CoV-2, ROX-labeled probe specific for N gene of SARS-CoV-2, and VIC-labeled probe specific for internal control. The internal control system is designed for a housekeeping gene as reference gene to assess the RNA quality and monitor the accuracy of experimental operation, which may lead to false negative results.
2) The **nCoV Enzyme Mix** contains the reverse transcriptase for reverse transcription of viral RNA and reference gene RNA into cDNA, the Taq DNA polymerase for PCR amplification and uracil-N-glycosylase to prevent PCR amplicon carryover contamination.
3) The **nCoV Positive Control** (PC) contains nucleic acid template of ORF1ab and N genes of coronavirus SARS-CoV-2 and housekeeping gene.

Kit contents

The kit contains the following materials (Table 1).

Table 1 Kit Contents

Contents	Main Ingredients	Quantity
nCoV Reaction Mix	Primers, Probes, Mg^{2+}, dNTPs	1050 μL/tube ×3
nCoV Enzyme Mix	Taq DNA Polymerase, Uracil-N-Glycosylase, Reverse Transcriptase	110 μL/tube ×1
nCoV Positive Control	Nucleic Acid Template	100 μL/tube ×1

Storage and Stability

The kit requires shipment on frozen ice packs. All contents of the kit should be stored immediately upon receipt at -20±5℃ and protected from light.

The shelf-life of the kit is eight months. Vial-opening does not affect the shelf-life of the kit. The maximal number of freeze-thaw cycles is five.

Additional Reagents and Equipment Required but Not Supplied

1) Compatible PCR instruments:
   Stratagene Mx3000P™, ABI7500 (Standard Module), LightCycler480 II, or SLAN-96S.
2) Viral RNA extraction kit: AmoyDx® Virus/Cell RNA Kit (Amoy Diagnostics, Cat. No.: 8.0250301X036G), or Nucleic Acid Isolation Kit (Da An Gene Co., Ltd, Cat. No.: DA0621).
3) Biosafety cabinet.

4) Vortexer.
5) Mini centrifuge with rotor for centrifuge tubes.
6) Personal Protective Equipment (PPE): Disposable powder-free gloves, goggles/face shield, respirators (NIOSH-certified N95 is recommended.)
7) Nuclease-free centrifuge tubes.
8) Nuclease-free PCR tubes and caps.
9) Adjustable pipettors and sterile, nuclease-free pipet tips.
10) Biohazard waste container.
11) Tube racks.
12) Sterile, nuclease-free water.

Precautions and Handling Requirements

For *in vitro* diagnostics use.

Precautions

- Please read the instruction carefully and become familiar with all components of the kit prior to usage. Strictly follow the instruction during operation.
- Please check the compatible real-time PCR instruments prior to usage.
- DO NOT use the kit or any kit component after their expiry date.
- DO NOT use any other reagents from different lots in the tests.
- DO NOT use any other reagent in the other test kits.

Safety Information

- Handle all specimens and components of the kit as potentially infectious material using safe laboratory procedures.
- Only trained professionals should use this kit. Wear appropriate personal protective equipment (PPE) when working with clinical specimens.
- Specimen processing should be performed in a certified Class II biosafety cabinet following biosafety level 2 or higher guidelines.
- Avoid contact of the skin, eyes and mucous membranes with the chemicals. In case of contact, flush with water immediately.
- DO NOT pipet by mouth.
- Avoid aerosols.

Decontamination and Disposal

- The kit contains PC; strictly distinguish the PC from other reagents to avoid contamination which may cause false positive results.
- PCR amplification is extremely sensitive to cross-contamination. The flow of tubes, racks, pipets, and other materials used should be from pre-amplification to post-amplification, and never backwards.
- Gloves should be worn and changed frequently when handling samples and reagents to prevent contamination.
- Use separate, dedicated pipettes and nuclease-free filtered pipette tips when handling samples and reagents to prevent exogenous RNA contamination to the reagents.
- Please pack the post-amplification tubes with two disposable gloves and discard properly. DO NOT open the post- amplification PCR tubes.
- All disposable materials are for one-time use. DO NOT reuse.
- The unused reagents, used kit, and waste must be disposed of properly.

Cleaning

- After the experiment, wipe down the work area, spray down the pipettes and equipment with 75% ethanol or 10% hypochlorous acid solution.

Instrument Setup

- Set up the reaction volume as 40 μL.

- For Stratagene Mx3000P™, please set up the Fliter Set Gain Settings of FAM, HEX and ROX as 2. If there's low net fluorescence signal (dR) but high background signal (R), please reduce the signal gain setting of instrument properly.
- For ABI 7500 (Standard Module), please set up as follows: Reporter Dye: FAM, VIC and ROX; Quencher Dye: NONE; Passive Reference: NONE.
- For SLAN-96S, please set up as follow: Probe mode: FAM, VIC and ROX. During the results analysis, open the "Preference" window, in "Chart Option" section, select "Selected Wells" for "Y-Axis Scaling Auto-adjust By" and "Absolute Fluorescence Value Normalization" for "Amplification Curve".
- For LightCycler480 II instrument, it's necessary to conduct Color Compensation prior to the first use according to Color Compensation instructions. To run the assay on a LightCycler machine, please use the Roche 480 adaptor, available from BIOplastics, Cat. No. B79480.
- Refer to the operations manual of the real-time PCR instrument for detailed instructions.
- We recommend that for all PCR instruments in use, a fluorescence calibration should be conducted once a year.

Assay Procedure

1. RNA Extraction

The specimen material must be viral RNA extracted from human respiratory specimens, including upper respiratory specimen (nasopharyngeal/oropharyngeal extracts, washes or swabs) and lower respiratory specimens (bronchoalveolar lavage samples, tracheal extracts or deep cough sputum). The viral RNA extraction reagents are not included in the kit.

Note:

- *Swab specimens should be collected only on swabs with a synthetic tip with aluminum or plastic shafts. Swabs with calcium alginate or cotton tips with wooden shafts are not acceptable.*
- *The samples should be extracted RNA immediately. For short-term storage of up to 24 hours, the sample should be stored at 4 ℃. For long-term storage of over 24 hours, the sample should be stored at -20 ℃ and avoid repeated freezing-thawing.*

2. Viral RNA Detection

1) Take the **nCoV Reaction Mix, nCoV Enzyme Mix** and **nCoV Positive Control** out of the kit from the freezer.
2) Thaw **nCoV Reaction Mix** and **nCoV Positive Control** at room temperature. When the reagents are completely thawed, invert each tube for 10 times and centrifuge briefly to collect the contents at the bottom of the tube.
3) Centrifuge the **nCoV Enzyme Mix** prior to use.
4) Prepare sufficient nCoV Master Mix containing nCoV Enzyme Mix and nCoV Reaction Mix in separate sterile centrifuge tube according to the ratio in Table 2. Mix nCoV Master Mix thoroughly by gently pipetting up and down more than 10 times, and then centrifuge briefly.

Table 2 nCoV Master Mix

Content	Volume per test
nCoV Reaction Mix	30 μL
nCoV Enzyme Mix	1 μL
Total	**31 μL**

Note:

- *Every PCR run must contain one PC (Positive control) and one No Template Control (NTC).*
- *Do not vortex enzyme mix or any mixture with enzyme mix.*
- *The prepared mixtures should be used immediately, avoid prolonged storage.*
- *Due to the viscosity of the enzyme mix, pipet slowly to ensure all mix is completely dispensed from the tip.*
- *Pipet enzyme mix by placing the pipet tip just under the liquid surface to avoid the tip being coated in excess enzyme.*

5) Take out the sample RNA and nuclease-free water for NTC.
6) Prepare PCR tubes for NTC/Sample/PC, dispense 31 μL nCoV Master Mix into each PCR tube respectively.

7) Add 9 μL NTC, 9 μL sample RNA or 9 μL **nCoV Positive Control** (nCoV-PC) to the appropriate PCR tubes. Cap the PCR tubes.

Note: *Suggest following below adding order: NTC → samples → PC, and use filtered tips for all pipetting steps to avoid cross-contamination.*

8) Briefly centrifuge the PCR strips to collect all liquid at the bottom of each PCR tube.

9) Place the PCR strips into the real-time PCR instrument. A recommended plate layout is shown in Table 3.

Table 3 Recommended PCR Plate Layout

Well	1	2	3
①	Sample1	Sample 9	Sample 17
②	Sample 2	Sample 10	Sample 18
③	Sample 3	Sample 11	Sample 19
④	Sample 4	Sample 12	Sample 20
⑤	Sample 5	Sample 13	Sample 21
⑥	Sample 6	Sample 14	Sample 22
⑦	Sample 7	Sample 15	nCoV-PC
⑧	Sample 8	Sample 16	NTC

10) Setup the PCR Protocol using the cycling parameters in Table 4.

Table 4 Cycling Parameters

Stage	Cycles	Temperature	Time	Data collection
1	1	55℃	15 min	/
		95℃	5 min	/
2	45	95℃	25 s	/
		55℃	35 s	FAM, HEX/VIC and ROX
		72℃	20 s	/
3	1	40℃	30 s	/

11) Start the PCR run immediately.

12) When the PCR run is finished, analyze the data according to the "Results Interpretation" procedures.

3. Results Interpretation

Before data analysis, the following items should be checked:

1) Analyze single fluorescent signal each time, and select the PC amplification curve to adjust the threshold value. Choose the reaction wells of PC, NTC and sample simultaneously for analysis.

2) For NTC: the FAM, ROX and HEX/VIC Ct values should be **> 45**. If not, the data are *INVALID*. The sample should be retested.

3) For nCoV Positive Control: the FAM, ROX and HEX/VIC Ct value should be **< 32**. If not, the data are *INVALID*. The sample should be retested.

Analyze the result for each sample:

4) Check the Internal control HEX/VIC signal for each sample:
   a. The HEX/VIC Ct value should be **≤ 40**.
   b. If the HEX/VIC Ct value is > **40**, it indicates any operation error, or partial RNA degradation or the presence of PCR inhibitors, the sample should be retested with increased or re-extracted RNA.

5) Check ORF1ab FAM and N ROX signals for each sample:
   a. If any Ct value (either ORF1ab FAM or N ROX Ct value) is **≤ 37**, the sample is determined as SARS-CoV-2 positive.
   b. If both Ct values (ORF1ab FAM and N ROX Ct value) are **> 40**, the sample is determined as SARS-CoV-2 negative or below the LOD.
   c. If both Ct values (ORF1ab FAM and N ROX Ct value) are **> 37**, and any one Ct value is **≤ 40**, retest the sample. If any Ct value (either ORF1ab FAM or N ROX Ct value) is still **≤ 40** in the retest result, the sample is determined as SARS-CoV-2 positive. If both Ct values (ORF1ab FAM and N ROX Ct value) are **> 40** in the retest result, the sample is determined as SARS-CoV-2

negative or below the LOD.

Performance Characteristics

The performance characteristics of this assay were validated on Stratagene Mx3000P™, ABI7500, LightCycler 480 II and SLAN-96S.

1) Limit of Detection (LOD): The LOD of this assay is 500 copies/mL.
2) Specificity:
   Specificity of the kit was established by testing 8 negative reference controls. The test gave negative results and negative concordance rate was 100%.
3) Accuracy:
   The accuracy of the kit was established by testing one positive reference control. The test gave positive result and positive concordance rate was 100%.
4) Precision:
   Precision of the kit was established by testing positive Precision Reference for 10 repeats; all the results should be positive and the coefficient of variation (CV, %) of Ct values is no more than 5%.
5) Interfering substance:
   Seven potential interfering substances: sodium chloride, hexadecadrol, ribavirin, levofloxacin, tobramycin, whole blood and mucus were evaluated in this study. It is confirmed that 45 μg/mL sodium chloride, 45 μg/mL hexadecadrol, 45 μg/mL ribavirin, 45 μg/mL levofloxacin, 45 μg/mL tobramycin, 5% whole blood, and mucus would not interfere with the test result.
6) Cross-reactivity:
   The kit has no cross-reactivity with 2009 Influenza A (H1N1), Respiratory adenovirus 3, Human Parainfluenza Virus 2, B/Victoria, Seasonal Influenza A (H1N1), Influenza A (H3N2), Influenza A (H7N9), Influenza A (H5N1), Human Coronavirus HKU1, Human Coronavirus 229E, and Human Coronavirus NL63.

Limitations

1) The kit is to be used only by personnel specially trained with PCR techniques.
2) Due to limited validation with clinical samples, the test results can be used to assist clinical diagnosis only, combining with other clinical and laboratory findings.
3) Reliable results are dependent on proper sample collection, processing, transportation, and storage.
4) The sample containing degraded RNA may affect the ability of the assay to detect novel coronavirus SARS-CoV-2.
5) The virus mutated in the ORF1ab and N target regions may affect the assay ability to detect novel coronavirus SARS-CoV-2.
6) The cross-reactivity with unknown virus may occur among the test.

Symbols

EC REP	Authorized Representative in the European Community	IVD	In Vitro Diagnostic Medical Device
	Manufacturer	REF	Catalogue Number
LOT	Batch Code		Use By
Σ	Contains Sufficient for <n> Tests		Temperature Limitation
	Consult Instructions For Use		Keep Dry
	This Way Up		Fragile, Handle With Care