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We use this protocol and it's working

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Isolation and Amplification of SARS-CoV-2 RNA from Nasopharyngeal Specimen

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DISCLAIMER

This is an optimised protocol for SARS-CoV-2 RNA isolation and amplification using Zymo Quick-RNA Viral Kit (200 prep) and Allplex 2019nCoV Assay Kit. The authors do not accept any liability for the collection and handling of both samples and reagents, results from the use of the protocol and its interpretation as well as any errors or omissions that may be made. The reader should make his/her own evaluation as to the appropriateness of the procedures described.

ABSTRACT

COVID-19 caused by the SARS-CoV-2 was declared a global pandemic by the World Health Organization in March 2020. Classical symptoms associated with the infection include fever, cough, chills, headache, and muscle aches amongst others. To effectively diagnose the infection and contain its spread, efficient diagnostic tools are required. The current gold standard for the confirmation of SARS-CoV-2 infection is RT-PCR, this protocol outlines procedures for the isolation and amplification of SARS-CoV-2 RNA from Nasopharyngeal specimens using the Zymo Quick-RNA Viral Kit and Allplex 2019nCoV Assay Kit respectively.

GUIDELINES

Viral RNA Wash Buffer Preparation
Viral RNA Buffer Preparation
RNA Isolation Procedure (Zymo Quick-RNA Viral Kit)
RT-PCR Mix Preparation Procedure (Allplex 2019nCoV Assay Kit)
RT-PCR Reaction Mix Preparation Procedure
RT-PCR Amplification Protocol (Allplex 2019nCoV Assay Kit)

Keywords: RNA, RNA Extraction, SARS-CoV-2, Zymo, COVID-19, RT-PCR, Virus, Nasopharyngeal specimen, Viral Transport Medium

MATERIALS

Reagent Preparation and RNA Extraction

Dithiothreitol (DTT)
Molecular grade ethanol (95-100%)
DNase/RNase-free water
Zymo Quick-RNA Viral Kit (Zymo Research, USA)
Cryo box
Automatic micropipette (10 µL, 20 µL, 200 µL and 1000µL)

RT-PCR

Allplex 2019nCoV Assay Kit (Seegen Inc., Korea)
1.5 mL - 2.0 mL tube ice rack
0.2 mL PCR tube ice rack

Consumables

0.5 - 10 µL filtered micropipette tips
2 - 20 µL filtered micropipette tips
20 - 200 µL filtered micropipette tips
100 - 1000 µL filtered micropipette tips
1.5 mL microcentrifuge tubes
Viral Transport Medium (Shanghai Focusgen Biotechnology Co., Ltd, China)
96 well qPCR plate and plate cover

Equipment

Centrifuge
Vortex
Biosafety Cabinet II
Bio-Rad CFX 1000 series

SAFETY WARNINGS

1. Perform all RNA isolation procedures in a Level II Biosafety Cabinet

2. Wear the appropriate PPEs before, during, and after the isolation and amplification of the SARS-CoV-2 RNA while still in the laboratory.

3. Dithiothreitol causes skin and eye irritation. Handle with care

4. Handle all reagents and specimens as potentially hazardous materials

BEFORE START INSTRUCTIONS

1. DTT is not stable in solution. Only freshly-made DTT solutions should be used

2. Aliquot the needed volume of reagents from the stock for the procedure to prevent contamination of the large reagent stock.

3. Allow the amplification kit to thaw completely in a 4°C fridge, before preparing the PCR master mix. Avoid centrifuging the reagents to defrost them.

4. Decontaminate all workspace with 1% bleach followed by 70% alcohol

Reagent Preparation - Viral RNA Wash Buffer

1 To each bottle of 48 mL of RNA Wash Buffer (concentrate) add 192 mL of molecular grade ethanol (95-100%).

2 Invert the reagent bottle several times and label with the date of preparation

Reagent Preparation - Viral RNA Buffer

3 Weigh 0.75 g of dithiothreitol (DTT) into 15 mL of nuclease-free water

4 Allow the pellets to dissolve completely.

5 Add 12 mL of the freshly prepared DTT solution to the 100 mL Viral RNA Buffer (concentrate). Invert several times to mix completely.

Note

Invert the reagent bottle gently to avoid over-frothing the reagent. Allow the reconstituted reagent to sit at room temperature for 60 seconds before use.

RNA Extraction Protocol (Zymo Quick-RNA Viral Kit)

6 Vortex the specimen for 00:00:30 at 3000 rpm and allow it to sit on the bench for 1 minute 30s

7 Aliquot 200 µL of the specimen into a sterile 1.5 mL microcentrifuge tube

8 Add to the specimen 400 µL of the RNA Buffer and vortex at 3000 rpm, 00:00:30 30s

9 Transfer the solution in step 3 into a Zymo-Spin™ IC Column in a clean collection tube and centrifuge at 8000 rpm, 00:02:00 2m

10 Discard the flow-through liquid and transfer the Zymo-Spin™ IC Column into a new collection tube.

11 Transfer into the Zymo-Spin™ IC Column 500 µL of RNA Wash Buffer (refer to reagent preparation for reconstitution of RNA Wash Buffer).

12 Centrifuge the Zymo-Spin™ IC Column in the collection tube at 10000 rpm, 00:00:30 and discard the flow-through liquid. Repeat this step. 30s

13 Add 500 µL of absolute ethanol to the Zymo-Spin™ IC Column and centrifuge at 10000 rpm, 00:01:00 and discard the flow-through liquid. 1m

14 Perform a dry-spin step at 13000 rpm, 00:01:00 rpm to eliminate any trace of ethanol. 1m

15 Transfer the Zymo-Spin™ IC Column into a new sterile 1.5 mL microcentrifuge tube and add to the spin column 25 µL of nuclease-free water.

16 Centrifuge the spin column for 1 minute at 8000 rpm. Cover the microcentrifuge tube while inspecting for the elute

17 Store the RNA at -20 °C pending downstream analysis.

Preparation of PCR Master Mix (Allplex™ 2019nCoV Assay ...

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	A	B	C
	Reagent	X 1 (µL)	X 10 (µL)
	2019 nCoV MuDT* Oligo Mix (MOM)	5.0	50.0
	RNase Free Water (PCR Grade)	5.0	50.0
	Real-time One-step Buffer	5.0	50.0
	Real-time One-step Enzyme	2.0	20.0
	Total Volume	17.0	170.0

MuDT is the brand name of Seegene's oligo mixture

NB:

1. Add the One-step Enzyme as the last reagent and pipette up and down several times to wash the reagent out of the tip since it is slightly viscous.

2. Pulse vortex the master mix and centrifuge at 3000 rpm for 30 seconds.

Preparation of RT-PCR Reaction Mix

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	A	B	C
	Reagent	X 1 (µL)	X 10 (µL)
	2019 nCoV MuDT* Oligo Mix (MOM)	5.0	50.0
	RNase Free Water (PCR Grade)	5.0	50.0
	Real-time One-step Buffer	5.0	50.0
	Real-time One-step Enzyme	2.0	20.0
	Viral RNA	8.0	-
	Total Reaction Volume	25.0	

MuDT is the brand name of Seegene's oligo mixture

NB:

1. Add 4 µL of the exogenous internal control (IC) to the extracted RNA immediately before pipetting the required volume for the reaction.

2. Centrifuge the qPCR plate or 8-strip qPCR tube for 30 seconds at 1000 rpm

RT-PCR Amplification Protocol (Allplex 2019nCoV Assay Kit)

20 **Cycling Conditions**: Reverse transcription at 50°C for 20 minutes, followed by an initial denaturation at 95°C for 15 minutes and 45 cycles of 94°C for 15 seconds and 58°C for 30 seconds (plate read: data acquisition)

Fluorophores: FAM (E gene), Cal Red 610 (RdRP gene), Quasar 670 (N gene), and HEX (Internal

Control).

Cut-off Ct-value: 40