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Quantiplus® CORONA VIRUS (2019nCoV) Detection KIT

(Real-time Qualitative PCR Kit)

Product Insert

QL-CNV-25 :25 rxnx
QL-CNV-50 :50 rxns

RUO

PI/QLCNV-00

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Introduction

Coronaviruses are a group of viruses that cause diseases in mammals, including humans, and birds. In humans, the virus causes respiratory infections which are typically mild but, in rare cases, can be lethal. There are no vaccines or antiviral drugs that are approved for prevention or treatment. Coronaviruses are viruses in the subfamily Orthocoronavirinae in the family Coronaviridae, in the order Nidovirales. Coronaviruses are enveloped viruses with a positive-sense single-stranded RNA genome and with a nucleocapsid of helical symmetry. The genomic size of coronaviruses ranges from approximately 26 to 32 kilobases, the largest for an RNA virus. Coronaviruses are believed to cause a significant percentage of all common colds in human adults and children. Coronaviruses cause colds with major symptoms, e.g. fever, throat swollen adenoids, in humans primarily in the winter and early spring seasons.[7] Coronaviruses can cause pneumonia, either direct viral pneumonia or a secondary bacterial pneumonia and they can also cause bronchitis, either direct viral bronchitis or a secondary bacterial bronchitis.

Product Description

This product contains Ready to Use Oligo mix for detection of Corona Virus, along with Reverse Transcriptase Enzyme for single tube RT qPCR amplification.

Recommended Work areas

Molecular Diagnostics work area includes:

a) Sample preparation area/room – for extraction of nucleic acids from clinical samples
b) Pre-PCR area/room - for setting up PCR reaction
c) PCR area/room – for performing PCR using the thermocyclers

As part of Good Laboratory Practices (GLP), it is recommended to have dedicated areas to avoid cross contamination.

General Precautions

Precautions while extracting Nucleic acid

Always wear proper attire (powder free gloves, facemask and Head cap) before starting the nucleic acid extraction procedure. During preparation of samples, compliance with good laboratory practices are essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of ribonucleases (RNases) into samples during and after the extraction procedure.

The Sample Preparation Area is dedicated to processing samples. All reagents used in the Sample Preparation Area should remain in this dedicated area at all times. Laboratory coats, pipettes, pipette tips and vortex mixer used in the Sample Preparation Area must remain in this area and not be moved to the Pre-PCR/PCR area. Discard the gloves before leaving this area. Do not bring amplified product into the Sample Preparation Area. Usage of filter tips is recommended while sample preparationshould be performed in a Biosafety cabinet.

Precautions while setting up a PCR reaction

PCR assay is sensitive and any accidental introduction of product from previous amplification reactions leads to incorrect results. Hence measures to reduce the risk of contamination in the laboratory should include physically separating the activities involved in performing PCR and complying with good laboratory practices.
It is recommended to have proper cleaning procedures to minimize the risk of cross contamination and carry over contamination (e.g. RNA OUT™, RNase OUT™, 0.1% Sodium Hypochlorite, Fumigation etc.).

Template is added in this area to the ready to use master mix. Laboratory coats and equipment used in the Pre-PCR Area must remain in this area and should not be moved to the Sample Preparation Area.

Precautions for post PCR or equipment area/room

The Real time PCR instrument/s should be kept in a segregated area away from Sample preparation area and Pre-PCR area.

Precautions after completion of Real time PCR assay

The reaction tubes or strips should be properly discarded without opening the caps, after the completion of run to avoid carry over contamination.

Usage Limitations

1. The kit and all its components are for *in-vitro* diagnostics use only.
2. The product is to be used by personnel specially trained in the *in-vitro* diagnostics procedures only.
3. Follow the product insert strictly for optimal PCR results.
4. Do not use the kit beyond the expiry date mentioned on the kit box.
5. Follow the guidelines provided in product insert for sample collection, storage and transport.
6. For ideal performance, store the kit under recommended conditions only.

Safety Precautions

1. All patient specimens should be considered as potentially infectious and handled in a BSL2 biosafety hood with BSL3 practices.
2. Wear personal protective equipment, including gloves, head cap, face mask and lab coats when handling kit reagents/sample extraction. Wash hands thoroughly using detergents before and after performing the test.
3. Do not smoke, drink or eat in areas where kit reagents and/or human specimens are being handled.
4. Dispose of unused kit reagents and human specimens as per regulatory guidelines.

Storage Conditions and Product Stability

1. All the kit reagents should be stored at -20°C. Replace all the kit components immediately at -20°C after usage.
2. Repeated thawing and freezing (more than 5 x) of all kit reagents should be avoided, as it reduces assay sensitivity. If needed, make aliquots of the kit reagents according to the volume used in the protocol prior to freezing.
3. Allow reagents to be thawed completely on Ice/4°C prior to use.
4. Kit reagents are stable through the end of the expiration date indicated on the box when stored at -20°C.

Symbols

Symbol	Meaning
	Catalog number
	Research Use Only
	Manufacturer
	Date of manufacture
	Contents sufficient for <n>tests
	Temperature limitations
	Use by date
	Batch number
	Consult Instructions for Use
	Important Note
	Biological risk (handle carefully)

Kit components

Color Coding (Caps)	Contents	Description	25 rxns (QL-CNV-25)	50 rxns (QL-CNV-50)
Amber	Huwel CoV Ready Mix	Oligo and Amplification Mix	1 x 375µL	1 x 750µL
Amber	Huwel 2019nCoV Ready mix	Oligo and Amplification Mix	1 x 375µL	1 x 750µL
Grey	Huwel IC-B Mix	Internal Control-B Mix	1 x 300µL	1 x 300 µL
Pink	Huwel RT Enzyme	cDNA Synthesis Reagent	1 x 25µL	1 x 50µL
Red	HUWEL nCoV PC	DNA Positive Control	1 x 50	1 x 100
White	Huwel PW	Purified water	1 x 0.5 mL	1 x 0.5 mL

Materials required but not supplied

The materials which are required but not supplied are listed below:

1. RNA Extraction kit
2. Biosafety Cabinet
3. PCR Hood
4. Calibrated variable micropipettes
5. Sterile pipette filter tips (aerosol free)
6. Vortex mixer
7. Dry Bath
8. Benchtop centrifuge with rotor for 1.5 mL reaction tubes
9. Real Time PCR machine
10. Spectrophotometer/Bio-analyzer
11. Strip Tubes and Caps (0.2 mL) or PCR Tubes (0.2 mL) or 96 well plate
12. Cooling block (96 x 0.2 mL tubes)
13. 1.5 mL centrifuge tubes
14. 1.5 mL centrifuge tube stand
15. Sterile powder free gloves
16. Facemask
17. Head cap
18. Lab coats

Quality Systems

In accordance with ISO-certified Quality Management System (9001:2008 and 13485: 2003) of HUWEL Lifesciences, each lot of Quantiplus® Corona Virus Qualitative PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Sample Type/Collection/Storage/Transport

Sample Type

nasopharyngeal swab, nasal aspirate or wash or a combined nasopharyngeal swab with oropharyngeal swab. If these specimens cannot be collected, a nasal swab or oropharyngeal swab is acceptable. For patients who are intubated, an endotracheal aspirate should also be collected. Bronchoalveolar lavage (BAL) and sputum specimens are also acceptable. Specimens should be placed into sterile viral transport media. and immediately placed on refrigerant gel-packs or at 4°C (refrigerator) for transport to the laboratory.

Sample Collection, Storage and Transport

Swab specimens should be collected using swabs with a synthetic tip (e.g., polyester or Dacron®) and an aluminum or plastic shaft. Swabs with cotton tips and wooden shafts are not recommended. Specimens collected with swabs made of calcium alginate are not acceptable. The swab specimen collection vials should contain 1-3ml of viral transport medium .(Sample should be immediately placed on refrigerant gel-packs or at 4°C (refrigerator) for transport to the laboratory. Respiratory specimens should be kept at 4°C for no longer than 3 days. Specimens can alternatively be frozen at ≤-70°C.

Sample Collection, Storage and Transport trough MTM (Molecular Transport Medium)

PrimeStore® Molecular Transport Medium (MTM) was designed to rapidly inactivate viruses (including Influenza), bacteria (including MTB) and high consequence veterinary pathogens (including ASFv, CSFv, FMDv, HPAI, and NDv) within the sample and stabilize the RNA and DNA to provide safer and more efficient workflow for molecular testing and sequencing. The cold chain is not needed for transportation from collection to the laboratory. It disrupts/lyses lipid membranes, destroys proteins and enzymes, inactivates nucleases and proteases, inactivates infectious biological pathogens, including gram-positive/negative bacteria and viruses, and preserves/stabilizes 'naked' RNA/DNA at elevated temperatures.

PrimeStore® MTM is compatible with most silica based spin column and magnetic bead extraction kits and systems to include those from Thermo Fisher, Roche Molecular, Qiagen, and BioMerieux. The 2019 MagMax Ultra kit kit may not provide optimized yields out of PrimeStore® MTM. Tested by **USDA** Foreign Animal Disease Diagnostic Laboratory

Assay Procedure

RNA Extraction

Quantiplus® CORONA VIRUS detection Kit has been validated using the following Viral RNA extraction kits:

1. QIAamp Viral RNA mini kit (cat no-52904)
2. Roche High Pure Viral Nucleic Acid kit (Cat. No. 11858874001)

Follow the manufacturer's instructions mentioned in the manual for Viral RNA extraction. Different pack sizes of the above mentioned kits can be used. However the customer can also validate their own extraction process using other Viral RNA extraction Kits.

S. No.	Name of the RNA Isolation Kit	Recommended Final Elution volume
1.	QIAamp Viral RNA mini kit (cat no-52904)	50 µL
2.	Roche High Pure Viral Nucleic Acid kit (Cat. No. 11858874001)	50 µL

Real time PCR Protocol

Preparation of Reaction Master mix

Quantitation procedure with Quantiplus® CORONA VIRUS detection Kit involves *1 step RT qPCR*. It is recommended that PC and a negative control(PW should be used as negative control) for each set of reaction are required to be included in a single run for obtaining proper results.

Set up a real time single step RTPCR reaction as follows:

1- *qPCR reaction setup*

Components	Volume per reaction in µL	Volume per reaction in µL
Huwel CoV Ready Mix	15.0	-
Huwel 2019nCoV Ready Mix	-	15.0
HUWEL RT Enzyme	1.0	1.0
Huwel IC-B Mix	1.0	1.0
Extracted RNA/ PW/PC	10.0	10.0
Total Volume	27.0	27.0

Place the PCR plate/tubes/strips in real time thermocycler.

PCR Programming

The QuantiplusHCV Quantitation kit is validated on the following instruments:

- Rotor-Gene™ 6000
- Rotor-Gene™Q 5plex
- ABI 7500 DX Real-Time PCR System
- ABI 7300 Real-Time PCR System
- Bio-Rad ™ CFX 96
- Quantstudio 3/5

Plate Setup

1. Program the plate setup by labeling the slots as per tube/strip/plate labels. The sequence of labeling of slots should be the same way as the tube/strip/plate is kept in the machine.
2. Select the type of sample (Unknown/PC/NTC) for each slot.
3. Select the channel for acquisition (FAM and HEX/VIC)

Name of channel	Source wavelength (nm)	Detection wavelength (nm)
FAM (Target)	470	510
HEX / VIC (IC)	530	555

4. For background calibration in different instruments, follow the procedure described below:

Rotor-Gene™ 6000	- Perform 'Gain optimization'
Rotor-Gene™Q 5plex	- Perform 'Gain optimization'
ABI 7500 DX Real-Time PCR System	- Select Passive Reference dye 'ROX' as none
ABI 7300 Real-Time PCR System	- Select Passive Reference dye 'ROX' as none
ABI Step One/Step One Plus	- Select Passive Reference dye 'ROX' as none
Thermo Quant Studio	- Select Passive Reference dye 'ROX' as none
Bio-Rad ™ CFX 96	- NA-

Cycling conditions

1. Configure the following program in the machine.

Steps	No. of cycles	Temperature (°C)	Time
1 (cDNA Synthesis)	1	42	30 min.
2 (Initial denaturation)	1	95	15 min.
3 (PCR cycling)	45	95	15 sec.
		58*	30 sec
*Plate Read/Data Acquisition in FAM and HEX / VIC Channel			

2. Set the reaction volume as 25 µL (the final volume is 26 µL but selecting 25 ul doesn't make any difference to the final result/sensitivity).
3. Plate read/Data Acquisition for FAM and HEX/VIC channel should be incorporated in the third stage of step 2 (55°C/30 sec).

Preparation of reaction master mix and cycling conditions are same for all the instruments listed in the product insert. For instrument specific protocols, please contact our technical support team at quantiplus@huwellifesciences.in

Data Analysis

Analyze the data after completion of the run. Check the R_n/*Cycle* amplification plot and ΔR_n/*Cycle* amplification plot to observe the amplification signal generated by different samples in the run. Compare both the plots for data analysis. Also look for noisy signals, if observed as it might not give you a proper result.

Setting the threshold for the qPCR Data analysis

The threshold should be set either automatically (by the machine itself)/ or manually just above the background signal of the negative controls and negative samples by referring to R_n/*Cycle* amplification plot. The mean threshold value calculated from these experiments will most likely work for the majority of future runs, but the user should nevertheless review the generated threshold value at regular intervals.

Result

The values for unknown samples would appear in the result column with *Ct* in FAM Channel. Samples showing no amplification with respective parameter should show amplification signal with IC-B mix in HEX/VIC channel, and the negative control should not show any value in the result column.
Then only results should be considered.

Interpretation

Interpret the values for unknown samples based on the observations as described in the following table and there should be no amplification in negative control. **IC** should show signal in all the samples to confirm RNA integrity of the sample.

CoV	***2019nCoV***	**Internal Control**	**Interpretation**	**Conclusion**
√	-	√	2019-nCoV, SARS-CoV and bat-SARS-related CoVs RNA Detected	Proceed for further Analysis
√	√	√	2019-nCoV RNA Detected	
-	-	√	nCoV related RNA Not Detected	
-	-	-	Possible inhibition of PCR	Dilute the RNA sample (1:10) and repeat the Assay

Troubleshoot

Observation	Possible cause	Solution
No amplification signal for PC in FAM channel	1. Incorrect channel selection 2. Incorrect programming of the real time machine. 3. Instrument is not working properly	1&2. Please recheck the PCR program 3. Contact manufacturer of Thermocycler for technical support.
Weak amplification signal for PC(Signal below threshold) in FAM Channel	1. Improper PCR programming. 2. Inaccurate dispensing of reagents 3. Possible deterioration of kit components due to improper storage	1. Repeat the assay by following the correct protocol 2. Minimize Pipetting errors/Check for calibration status of pipettes

Identical/Similar Ct values observed in FAM channel	1. Possible contamination of Kit reagents/PC/Work area.	1. Use fresh aliquots of Standards/Kit Reagents (if available) 2. Clean the PCR rack/Pipettes thoroughly as per GLP 3. Clean and Fumigate the work area overnight prior to use

References

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For any other technical query; please contact quantiplus@Huwellifesciences.com

Ordering Information

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