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Quantiplus® Influenza RT-PCR KIT

(Real-time Qualitative PCR Kit)

Product Insert

QL-INF-25 :25 rxnx
QL-INF-50 :50 rxns

RUO

PI/QLINFV-00

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Product Description

This product contains single tube probe primer mixes for detection of influenza-A, Influenza-B, swine H1 influenza and Swine H3N2 influenza along with RT mix for single tube reaction 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-INF-25)	50 rxns (QL-INF-50)
Amber	Huwel InfA Ready Mix	Oligo and PCR Mix	1 x 375µL	1 x 750µL
Amber	Huwel InfB Ready mix	Oligo and PCR Mix	1 x 375µL	1 x 750 µL
Amber	Huwel H1N1 Ready mix	Oligo and PCR Mix	1 x 375µL	1 x 750 µL
Amber	Huwel H3N2 Ready mix	Oligo and PCR Mix	1 x 375µL	1 x 750 µL
Amber	Huwel ICP Ready mix	Oligo and PCR Mix	1 x 375µL	1 x 750 µL
Pink	Huwel RT Enzyme	cDNA synthesis reagent	1 x 125µL	2 x 125µL
White	Huwel PW	Purified water	1 x 0.5 mL	1 x 0.5 mL
Red	InfA PC	DNA Positive Control	1 x 50	1 x 100
Red	InfB PC	DNA Positive Control	1 x 50	1 x 100
Red	H1N1 PC	DNA Positive Control	1 x 50	1 x 100
Red	H3N2 PC	DNA Positive Control	1 x 50	1 x 100

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®Influenza 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.

Assay Procedure

RNA Extraction

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

1. QIAamp Viral RNA mini kit (cat no-52904)

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.

Real time PCR Protocol

Preparation of Reaction Master mix

Quantitation procedure with Quantiplus® influenza 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 (µL) (for final vol. of 26 µL)
Huwel InfA/InfB/H1N1/H3N2/ICP Ready Mix	15.0
Huwel RT Enzyme	1.0
Extracted RNA/ PC/ PW	10.0

2- ***Reactions required per sample:***

Parameter	InfA	*InfB*	*H1N1*	*H3N2*	*ICP*
Reactions required	*Sample*	*Sample*	*Sample*	*Sample*	*Sample*
	PC	*PC*	*PC*	*PC*	--
	NTC	*NTC*	*NTC*	*NTC*	*NTC*

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)

S. No.	Name of channel	Source wavelength(nm)	Detection wavelength (nm)
1.	FAM (Pathogen target and ICP)	470	510

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.
		55*	30 sec
*Plate Read/Data Acquisition in FAM Channel			

1. 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).
2. Plate read/Data Acquisition for FAM and Yakima Yellow/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 ICP mix, 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. ICP should show signal at 37 or lower Ct to confirm RNA integrity of the sample.
Any amplification crossing threshold after 40 Ct should be considered Negative.

Inf A	*Inf B*	*H1N1*	*H3N2*	*ICP*	Interpretation	Conclusion
√	-	-	-	√	Inf A RNA Detected	Proceed for further Analysis
-	√	-	-	√	Inf B RNA Detected	
√	-	√	-	√	Inf A,H1N1 RNA Detected	
√	-	-	√	√	Inf A,H3N2 RNA Detected	
-	-	-	-	√	Influenza 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

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

Ordering Information

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QL-INF-50 : 50 rxns