Quantiplus® Multiplex FLU RT PCR Kit (Real-Time Qualitative PCR Kit)

QL-FLU-25 : 25 rxn
QL-FLU-50 : 50 rxn

RUO

PI/HL/QLFLU-05

Introduction

Quantiplus® Multiplex FLU RT PCR Kit (Real-Time Qualitative PCR Kit) is a diagnostic test, based on Real-Time PCR technology, for the qualitative detection and differentiation of Influenza A virus, Influenza B virus, Influenza A (H1N1) and Influenza A (H3N2) virus specific RNA.

Product description

Quantiplus®Multiplex FLU RT PCR Kit (Real-Time Qualitative PCR Kit) is a reverse transcription-Real Time PCR based *in-vitro* diagnostic assay for detection and differentiation of Influenza A serotypes including H1N1, H3N2 and Influenza B lineages Victoria and Yamagata in human throat or Nasal swabs. The kit contains target specific Primer Probe Mix for pathogen & Endogenous Internal Control, Positive Control, MBGPW and R Fastcore qPCR Mix (2X) with Reverse Transcriptase, QuickStart Taq polymerase enzyme and UDG/UNG enzyme. This advanced formulation enables performance of Fast PCR in shorter runtime and UDG/UNG helps in controlling PCR carryover contamination.

Kit components

Color Coding (Caps)	Contents	Description	25 rxns (QL-FLU-25)	50 rxns (QL-FLU-50)
Green	R Fastcore qPCR Mix (2X)	PCR Amplification Mix	3 x 325 µL	3 x 650 µL
Amber	InfD PPM	Primer Probe Mix for Influenza A and B	1 x 50 µL	1 x 100 µL
Amber	Inf H1N1 PPM	Primer Probe Mix for H1N1	1 x 50 µL	1 x 100 µL
Amber	Inf H3N2 PPM	Primer Probe Mix for H3N2	1 x 50 µL	1 x 100 µL
Red	InfD PC	Positive Control for Influenza-A and B	1 x 100 µL	1 x 100 µL
Red	Inf H1N1 PC	Positive Control for H1N1	1 x 100 µL	1 x 100 µL
Red	Inf H3N2 PC	Positive Control for H3N2	1 x 100 µL	1 x 100 µL
White	MBGPW	Molecular Biology Grade Purified water	2 x 500 µL	2 x 1 mL

Storage and Transportation Conditions

The kit should be transported at temperatures below −20 °C. The kit is stable until the expiry date printed on the package, if the storage temperature is within −20 ±5 °C. More than 4X freezing and thawing cycles reduces the assay sensitivity. For intermittent usage the reagents should be frozen in aliquots.

Technical Specification

Specificity	Influenza A, H1N1, H3N2 and Influenza B with 100% specificity
Sensitivity	6 copies/PCR
Validated Specimen	Nasal/Throat swabs, Nasal aspirates, BAL, Sputum and Endotracheal wash
External Quality Assessment	QCMD EQA Panels

Assay Procedure

RNA Extraction

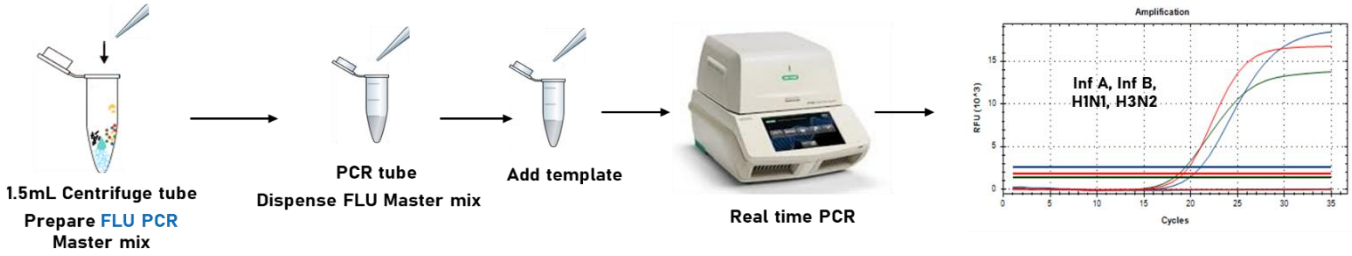
Quantiplus®Multiplex FLU RT PCR Kit (Real-Time Qualitative PCR Kit) has been validated using the following Viral RNA extraction kits:

Recommended sample volume for extraction and elution are as follows:

S. No.	Name of the Extraction Kit	Recommended Sample volume for Extraction	Recommended Final Elution volume
1.	QIAamp Viral RNA Mini Kit (Cat. No. 52904)	140 µL	60 µL
2.	Huwel Nucleic Acid Extraction Kit-version 2.0 (Cat. No. HL-NAX-100)	200 µL	100 µL

Note: Customer can also validate their own extraction process using other Viral RNA extraction Kits.

qPCR Protocol Flow



Preparation of Reaction Master mix

Components	Volume per reaction (for 25µL)
R Fastcore qPCR Mix (2X)	13
InfD / Inf H1N1/ Inf H3N2 PPM	2.0
Extracted RNA/ InfD PC/ Inf H1N1 PC/ Inf H3N2 PC/ MBGPW	10.0

It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation. Close the tubes, centrifuge shortly before processing in to thermal cycler.

Cycling Conditions

Steps	No. of cycles	Temperature (°C)	Time
1(Reverse Transcription)	1	53	5 min.
2 (Initial denaturation)	1	95	2 min.
3 (PCR cycling)	40	95	15 sec.
		56*	30 sec.
*Plate Read/Data Acquisition in FAM, HEX/VIC,and Texas Red/ROX Channel			

Sample analysis and 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 at 36 or lower Ct to confirm RNA integrity of the sample. Any amplification crossing threshold after 38 Ct should be repeated with freshly collected sample for confirmation

Influenza Differentiation (Influenza A and B)			
Inf A (HEX/VIC)	Inf B (Texas Red/ROX)	Interpretation	Conclusion
√	√	Influenza A&B Positive	Proceed for further Analysis
√	-	Influenza A Positive	
-	√	Influenza B Positive	
-	-	Influenza A&B Negative	

For H1N1			
H1N1 (pdm 2009) (FAM)	RNase P (TEXAS RED/ROX)	Interpretation	Conclusion
√	√	H1N1 positive	Proceed for further Analysis
-	√	H1N1 negative	
√	-	If FAM channel (H1N1) shows strong signals, ignore the RNase P result, and consider sample as H1N1 positive.	
-	-	Possible inhibition of PCR	Re-Test with freshly Isolated RNA or dilute to 1:10 and retest

For H3N2			
H3N2 (FAM)	RNase P (TEXAS RED/ ROX)	Interpretation	Conclusion
√	√	H3N2 positive	Proceed for further Analysis
-	√	H3N2 negative	
√	-	If FAM channel (H3N2) shows strong signals, ignore the RNase P result, and consider sample as H3N2 positive.	
-	-	Possible inhibition of PCR	Re-Test with freshly Isolated RNA or dilute to 1:10 and retest

Note : All the Target channels (FAM,HEX, and Texas Red) to be analyzed individually as described below

Channels to be analyzed			Parameters
(FAM)	(HEX/VIC)	(Texas Red/ROX)	
√			Analyze for H1N1
√			Analyze for H3N2
	√		Analyze for Inf A
		√	Analyze for Inf B in InfD PPM
		√	Analyze for RNase P in H1N1 and H3N2 PPM

Validated Instruments

- Thermo QS5 Real-Time PCR System
- Bio-Rad ™ CFX 96

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