A Multiplex Real-Time PCR (qPCR) Assay for Detection of Four Influenza (FLU) Types

Product Insert

*ab*TES™ FLU 4 qPCR V Kit
Kit Version: 4.0

CE IVD

REF 300113 (50 Reactions)
300114 (100 Reactions)

Store at -25°C to -15°C

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For use on Bio-Rad CFX96 and Stratagene Mx3005P.

1. Pathogen Information

Influenza remains one of the most crucial health problems throughout the world. Influenza A (FluA) and Influenza B (FluB) viruses is a genus of the orthomyxovirade family of virus. An infection with FluA and FluB can sometimes cause pneumonia, which can be fatal particularly for the young and the elderly.

FluA comprises of many subtypes categorised according to the type of hemagglutinin (H) and neuraminidase (N) present. Wild birds are the natural hosts for a large variety of FluA subtypes. Occasionally, viruses are transmitted to other species and may then cause devastating outbreaks in domestic poultry or give rise to human influenza pandemics. Currently, the most common subtypes of FluA that are circulating among humans are the seasonal H1N1 (huH1N1), H3N2 (huH3N2) and the pandemic H1N1/2009 (swH1N1) including the newly mutated strain which causes an outbreak in India in 2015[1, 2].

2. Test Description

The *ab*TES™ FLU 4 qPCR V Kit is a multiplex real-time polymerase chain reaction (qPCR) kit for the simultaneous detection of **FluA, FluB, swH1N1 and huH3N2** in one reaction tube. The kit contains all the necessary PCR reagents for rapid, sensitive and reproducible real-time detection of four Influenza types using highly specific primer pairs and double-dye hydrolysis probes. The recommended human sample types are nasal and throat swabs.

3. Storage Conditions

The components of *ab*TES™ FLU 4 qPCR V Kit should be stored in the dark, at -20 °C in a **NON**-frost-free freezer. Frost-free freezers go through freeze-thaw cycles to remain frost-free and may cause accelerated degradation of enzymes and nucleic acids. Avoid repeated thawing and freezing (max 3 times) as this may lower the sensitivity. If reagents will be used intermittently, it is suggested to keep the reagents frozen in aliquots.

4. Kit Components

Tubes	Items	300113 (50 rxns)	300114 (100 rxns)
1	2x RT-PCR Reaction Mix	1x 625 µL	2 x 625 µL
2	RT/*Taq* Enzyme Mix	1x 25 µL	2 x 25 µL
3	Primer/Probe Mix	1x 50 µL	2 x 50 µL
4	Nuclease-free Water	1x 600 µL	2 x 600 µL
5	FLU ALL-PC	1x 50 µL	1x 50 µL

5. Additional Materials Required but not Provided

- Disposable powder-free gloves
- Nucleic acid extraction kit
- Vortex mixer
- Pipettes and pipette tips with filter
- Desktop centrifuge with rotor
- Real-time thermal cycler
- 0.2 mL PCR tubes/ 96-well PCR plates
- Ice box/cooling block

6. Limitations and General Precautions

- The use of this product and its data interpretation are intended for personnel trained in real-time PCR techniques and *in vitro* diagnostics procedures only.
- It is advisable to analyze the real time PCR graph at the end of the run to determine the validity of the Ct data.
- Appropriate specimen collection, transport, storage and nucleic acid extraction procedures are required for reliable results.
- Wear disposable gloves, laboratory coats and eye protection when handling samples and reagents. Wash hands thoroughly thereafter.
- Use sterile pipette tips with filters and replace the tip for every procedure.
- Store and extract positive materials (specimens, controls and amplicons) separately from all other reagents and add to the reaction mix in a separate facility, if possible.
- Thaw all the components thoroughly at room temperature before starting an assay.
- When the components are thawed, mix the components and centrifuge briefly.
- Do not use the kit after its expiration date.

7. Procedures

7.1 Nucleic Acids (NA) Extraction

Standard NA extraction kits are compatible with this assay. Please carry out NA extraction as per instructed in the manufacturer's extraction kit manual.

7.2 PCR Reaction Setup

Thoroughly thaw all components, mix and spin briefly. Keep all components and samples on ice. Prepare your PCR reaction based on the following pipetting scheme:

Reagent	Volume per reaction		
	Test sample reaction	Positive control reaction	Non template control reaction
2x RT-PCR Mix	12.5 µL	12.5 µL	12.5 µL
RT/ *Taq* Enzyme Mix	0.5 µL	0.5 µL	0.5 µL
Primer/Probe Mix	1 µL	1 µL	1 µL
Nuclease-Free Water	8.5 µL	8.5 µL	11 µL
Positive Control	-	2.5 µL	-
Extracted Test Sample	2.5 µL	-	-
Total Volume	**25 µL**	**25 µL**	**25 µL**

the lowest concentration at which the assay can detect with a positivity rate of at least 95%. The analytical sensitivity of the assay was determined by analyzing a dilution series of Commercial RNA Controls (Vircell) from 0.01 or 0.001 to 1000 copies/PCR. The reactions were performed in triplicates on 6 different days (total of 18 replicates per dilution). The analytical sensitivity was estimated by probit analysis using SPSS release 16.0.0.

The analytical sensitivity (not in consideration of extraction) was determined to be the following:

Target	CFX96 Channel	Analytical Sensitivity (95% confidence)
swH1N1	HEX	0.22 copies/µl
FluA	FAM	0.41 copies/µl
FluB	Texas Red	5.92 copies/µl
huH3N2	Cy5	0.29 copies/µl

8.1. Analytical Specificity

The assay was tested for potential cross-reactivity against the following panel of 24 organisms. No cross-reactivity was observed.

Organisms Tested for Analytical Specificity	
Bordetella pertussis	Neisseria meningitidis
Corynebacterium diphtheriae	Parainfluenza 1
Coxsackie a6	Parainfluenza 2
Coxsackie b1	Parainfluenza 3
Coxsackie b5	Pseudomonas aeruginosa
Escherichia coli (vtec)	Staphylococcus aureus
Haemophilus influenza	Streptococcus pyogenes
Hu Adenovirus 1	Influenza A Pandemic H1N1/2009 (NIBRG-122)
Hu respiratory syncytial (A)	Influenza A H3N2 (A/VICTORIA/3/75)
Hu respiratory syncytial (B)	Influenza B (B/HONG KONG/5/72)
Klebsiella pneumoniae	Influenza A H1N1 (A/PR/8/34)
Legionella pneumophila	Streptococcus pneumoniae

8.3 Precision/Reproducibility

The inter-assay and intra-assay precision was determined by performing the assay once per day in triplicates over a period of 6 days for 4 samples with different concentrations (total of 72 reactions per target). To also account for variability between technicians, the testing over the 6 days were tested by 2 different technicians.

For all the 4 targets, the qualitative results of all 72 reactions were 100% reproducible for both inter-assay and inter-technician.

The coefficient of variation (CV) of the cycle threshold (Ct) for the intra- and inter-assay precision are as follows:

Inter-assay precision data showing %CV (calculated from Ct values) at each concentration:

	400 copies/µl	40 copies/µl	20 copies/µl	4 copies/µl
swH1N1	1.3%	1.2%	1.6%	1.7%
FluA	1.6%	1.1%	2.0%	1.5%
FluB	1.6%	1.8%	1.5%	2.4%
huH3N2	1.7%	2.0%	1.7%	1.7%

Intra-assay precision data showing %CV (calculated from Ct values) at each concentration:

	400 copies/µl	40 copies/µl	20 copies/µl	4 copies/µl
swH1N1	0.9%	0.6%	0.6%	1.0%
FluA	1.2%	0.7%	0.6%	1.1%
FluB	0.7%	1.0%	0.9%	1.7%
huH3N2	0.7%	0.5%	0.5%	0.7%

8.4 Diagnostic Evaluation

A retrospective study was conducted with 218 clinical samples for diagnostic evaluation. 55 samples of FluA (swH1N1), 63 samples of FluA (huH3N2), 50 samples of FluB and 50 Flu negative samples were tested and the outcome was matched for concordance with the previously typed result. The diagnostic sensitivity and specificity for each influenza virus type is summarized in below tables.

Diagnostic sensitivity and specificity of **FluA**:

Reference Method	*ab*TES (*n*=218)		Sensitivity/ Specificity
	FluA Positive	FluA Negative	
FluA Positive	118	0	100% sensitivity
FluA Negative	0	100	100% specificity
Total	118	100	

Diagnostic sensitivity and specificity of **swH1N1**:

Reference Method	*ab*TES (*n*=218)		Sensitivity/ Specificity
	swH1N1 Positive	swH1N1 Negative	
swH1N1 Positive	55	0	100% sensitivity
swH1N1 Negative	1	162	99.38% specificity
Total	56	162	Total

Diagnostic sensitivity and specificity of **FluB**:

Reference Method	*ab*TES (*n*=218)		Sensitivity/ Specificity
	FluB Positive	FluB Negative	
FluB Positive	50	0	100% sensitivity
FluB Negative	0	168	100% specificity
Total	50	168	Total

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Diagnostic sensitivity and specificity of **huH3N2**:

Reference Method	*ab*TES (*n*=218)		Sensitivity/ Specificity
	huH3N2 Positive	huH3N2 Negative	
huH3N2 Positive	63	0	100% sensitivity
huH3N2 Negative	0	155	100% specificity
Total	63	155	

8. Interpretation of Data

A sample will be considered as having a positive result if an amplification signal is detected in the respective fluorescence channel.

Result	FluA (FAM)	swH1N1 (HEX)	FluB (TxR)	huH3N2 (Cy5)
FluA positive	+	-	-	-
swH1N1 positive	+	+	-	-
FluB positive	-	-	+	-
huH3N2 positive	+	-	-	+
FluA and FluB negative	-	-	-	-

9. Troubleshooting

10.1. No signal observed with positive control

- Check programmed temperature settings against the protocol given.
- Affirm if proper storage was done and check the expiry date on the kit; use a newer kit to repeat experiments if needed.
- PCR inhibition has possibly occurred: check to ensure the preservation of purity of the components of the kit is maintained and no presence of PCR inhibitors in reaction setups/consumables used. Repeat PCR if needed.

10.2. Signal detected for negative control

- A contamination in the reagents is highly possible.
- Repeat experiment protocol and take steps to locate source of contamination.

11. Explanation of Symbols

IVD	*In vitro* diagnostic medical device
REF	Catalogue number
	Store at -25°C to -15°C
	Manufacturer
EC REP	Authorized representative in the European community
LOT	Lot number
	Use by
	Contains sufficient for <n> tests

12. References

1. https://en.wikipedia.org/wiki/2015_Indian_swine_flu_outbreak

2. http://news.mit.edu/2015/swine-flu-outbreak-india-mutations-0311

Electronic copy of the product insert can be downloaded at http://aitbiotech.com/influenza/

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