A Multiplex Real-Time PCR (qPCR) Assay for Detection of Five Influenza (Flu) Types

Product Insert

*ab*TES™ FLU 5 qPCR I Kit
Kit Version: 4.0

RUO For research use only

REF 300101 (50 Reactions)
300102 (100 Reactions)

Store at -25°C to -15°C

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For use on Bio-Rad CFX96.

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 5 qPCR I Kit is a multiplex real-time polymerase chain reaction (qPCR) kit for the simultaneous detection of **FluA, FluB, swH1N1, huH1N1 and huH3N2** in one reaction tube. The kit contains all the necessary PCR reagents for rapid, sensitive and reproducible real-time detection of all five Influenza types using highly specific primer pairs and double-dye hydrolysis probes. The recommended human sample types are nasal swabs, wash and aspirate.

3. Storage Conditions

The components of *ab*TES™ FLU 5 qPCR I 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	300101 (50 rxns)	300102 (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**

7.3 PCR Cycling Conditions

The following cycling conditions were established and validated on Bio-Rad CFX96. You may need to adjust these conditions for other real-time platforms. **FAM** (FluA), **Texas Red** (FluB), **HEX** (swH1N1), **Quasar 705** (huH1N1) and **Cy5** (huH3N2) channels should be chosen and the fluorescence is measured at the annealing phase of each cycle.

Phase	Description	No. of Cycles	Temperature	Duration
1	cDNA synthesis	1	55 °C	10 min
2	*Taq* activation	1	95 °C	2 min 30 sec
3	Amplification	42	95 °C	17 sec
			*55 °C	31 sec
			68 °C	32 sec

**Data acquisition at annealing phase*

8. Interpretation of Data

A sample will be considered as having a positive result if the fluorescence level is higher than the threshold value and will be considered negative, otherwise.

Result	FluA (FAM)	swH1N1 (HEX)	FluB (TxR)	huH3N2 (Cy5)	huH1N1 (Q705)
FluA positive	+	-	-	-	-
swH1N1 positive	+	+	-	-	-
FluB positive	-	-	+	-	-
huH3N2 positive	+	-	-	+	-
huH1N1 positive	-	-	-	-	+
FluA and FluB negative	-	-	-	-	-

9.0 Troubleshooting

9.1 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
- Check proper volume of the reagent added during the PCR setup.

9.2 Signal detected for negative control

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

10.0 Explanation of Symbols

RUO	For research use only
REF	Catalogue number
-15°C / -25°C	Store at -25°C to -15°C
	Manufacturer
LOT	Lot number
	Use by
Σ	Contains sufficient for <n> tests

11.0 References

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

2. http://news.mit.edu/2015/swine-flu-outbreak-india-mutations-03

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

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