A Real-Time PCR (qPCR) Assay for Detection of Influenza (Flu) H7N9

Product Insert

*ab*TES™ H7N9 qPCR I Kit
Kit Version: 1.1

RUO For research use only

REF 300125 (50 Reactions)
300126 (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 A (FluA) is one of the three kinds of Influenza virus that infects influenza in birds and mammals. It comprises of many subtypes categorized according to the type of hemagglutinin (H) and neuraminidase (N) present.

Avian influenza A(H7N9) is a subtype of influenza viruses that have been detected in birds in the past but it was until March 2013 that infections in both humans and birds are observed in China. The disease is of concern because most patients have become severely ill.

Most of the cases of human infection with this avian H7N9 virus have reported recent exposure to live poultry or potentially contaminated environments, especially markets where live birds have been sold. This virus does not appear to transmit easily from person to person, and sustained human-to-human transmission has not been reported.

2. Test Description

The *ab*TES™ H7N9 qPCR I Kit is a real-time polymerase chain reaction (qPCR) kit for the detection of H7N9. The kit contains all the necessary PCR reagents for rapid, sensitive and reproducible real-time detection of H7N9 using highly specific primer pairs and double-dye hydrolysis probes. The recommended human sample types are nasal swabs, wash and aspirate. An **Internal Control (IC)** is also supplied to check for possible PCR inhibition.

3. Storage Conditions

The components of *ab*TES™ H7N9 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	300125 (50 rxns)	300126 (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	Internal Control	1x 200 µL	2 x 200 µL
5	H7N9 PC	1x 50 µL	1x 50 µL
6	Nuclease-free Water	600 µL	2 x 600 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	Negative control reaction	Non template reaction
2x RT-PCR Mix	12.5 µL	12.5 µL	12.5 µL	12.5 µL
RT/ *Taq* Enzyme Mix	0.5 µL	0.5 µL	0.5 µL	0.5 µL
Primer/Probe Mix	1 µL	1 µL	1 µL	1 µL
Nuclease-Free Water	8.4 µL	8.4 µL	10.9 µL	11 µL
Internal control	0.1 µL	0.1 µL	0.1 µL	-
Positive Control	-	2.5 µL	-	-
Extracted Test Sample	2.5 µL	-	-	-
Total Volume	**25 µL**	**25 µL**	**25 µL**	**25 µL**

7.3 PCR Cycling Conditions

The following cycling conditions were established and validated on Bio-Rad CFX96 and Stratagene Mx3005P. You may need to adjust these conditions for other real-time platforms. **FAM** (H7N9) and **HEX** (IC) 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.

9. 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.

9.3. Weak or no signal of the internal control and no sign detection in analytical channel as well

- A possible PCR inhibition has occurred. Re-extract the sample to remove inhibitors and repeat PCR, if needed.

Affirm if proper storage was done and check the expiry date on the kit. Use a new kit to repeat the test if needed.

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9.4. Explanation of Symbols

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

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

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