A Multiplex Real-Time PCR (qPCR) Assay for Detection of Influenza A & B and Respiratory Syncytial virus A & B

Product Insert

*ab*TES™ FLU/RSV qPCR I Kit
Kit Version: 2.0

RUO For research use only

REF 300267 (50 Reactions)
300268 (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.

Influenza A viruses are negative-sense, single-stranded, segmented RNA viruses comprise of many subtypes categorized 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.
Influenza B virus does not have any subtypes, but it can be further broken down into lineages and strains. The currently circulating influenza B viruses belong to one of two lineages: B/Yamagata and B/Victoria. The FluB viral genome is 14,548 nucleotides long and consists of eight segments of linear negative-sense; single-stranded RNA. It is only known to infect humans and seals. This limited host and range is apparently responsible for the lack of Influenza virus B-caused influenza pandemics in contrast with those caused by the morphologically similar Flu A viruses as both mutate by both antigenic drift and reassortment.

Human respiratory syncytial virus (RSV) is a virus that causes respiratory tract infections. It is a major cause of lower respiratory tract infections and hospital visits during infancy and childhood. RSV is negative sense, single stranded RNA viruses of the family Paramyxoviridae. RSV is subdivided into two major genetic groups, RSV A and RSV B. The viral genome is 15,000 nucleotides in length and is composed of a single strand of RNA with negative polarity.

In most cases RSV infection causes only mild symptoms, like common cold. But in infants and young children RSV is the most important cause of severe respiratory illness and the major cause of infantile bronchiolitis and pneumonia. In addition, RSV is a significant problem in the elderly, in persons with cardiopulmonary diseases and in immunocompromised individuals.

2. Test Description

The *ab*TES™ FLU/RSV qPCR I Kit is a multiplex real-time polymerase chain reaction (qPCR) kit for the simultaneous detection of **FluA, FluB, RSVA and RSVB** in one reaction tube. The kit contains all the necessary PCR reagents for rapid, sensitive and reproducible real-time detection of all four Influenza viruses using highly specific primer pairs and double-dye hydrolysis probes. The recommended human sample types are nasal swabs, wash and aspirate. The *ab*TES™ FLU/RSV qPCR I Kit enables detection of the mRNA of human housekeeping gene, GAPDH as an **Internal Control (IC)** to identify possible PCR inhibitions and sampling problems.

3. Storage Conditions

The components of *ab*TES™ FLU/RSV 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	300267 (50 rxns)	300268 (100 rxns)
1	2X RT-PCR Reaction Mix	1 x 500 µL	2 x 500 µL
2	RT Enzyme Mix	1 x 50 µL	2 x 50 µL
3	Primer/Probe Mix	1 x 100 µL	2 x 100 µL
4	FLU/RSV Positive Control	1x 100 µL	1x 100 µL
5	Nuclease-free Water	1 x 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
2x RT-PCR Mix	10 µL	10 µL	10 µL
RT/ *Taq* Enzyme Mix	1 µL	1 µL	1 µL
Primer/Probe Mix	2 µL	2 µL	2 µL
Nuclease-Free Water	2 µL	2 µL	7 µL
Positive Control	-	5 µL	-
Extracted Test Sample	5 µL	-	-
Total Volume	**20 µL**	**20 µL**	**20 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), **ROX** (FluB), **CY5** (RSVA), **Quasar 705** (RSVB) and **HEX (**IC) channels should be chosen and the fluorescence is measured at the annealing phase of each cycle in amplification 2 phase.

Phase	Description	No. of Cycles	Temperature	Duration
1	cDNA synthesis	1	55 °C	10 min
2	*Taq* activation	1	95 °C	2 min
3	Amplification	5	95 °C	10sec
			55 °C	30 sec
	Amplification	40	95 °C	10sec
			* 55 °C	20 sec
			72 °C	10 sec

**Data acquisition at annealing phase*

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.

The result is regarded as true negative for Influenza A and B or RSV A and B when the FAM, ROX, Cy5 and Q705 channels are negative and the HEX Internal Control channel is positive.

Target	FluA (FAM)	FluB (ROX)	RSVA (Cy5)	RSVB (Q705)	IC - GAPDH (HEX)
FluA positive	+	-	-	-	+ / -
FluB positive	-	+	-	-	+ / -
RSVA positive	-	-	+	-	+ / -
RSVB positive	-	-	-	+	+ / -
Flu and RSV negative	-	-	-	-	+
Positive Control	+	+	+	+	-
Non-template Control	-	-	-	-	-

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
- PCR inhibition has possibly occurred: re-purify DNA sample to remove inhibitors and repeat PCR, if needed

9.2 Signal detected for negative control

- A contamination in the reagents or samples 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 signal detected in analytical channel as well

- A contamination in the reagents or samples is highly possible. Repeat experiment protocol and take steps to locate source of contamination
- 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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10. 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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