

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

Product Insert
abTES™ FLU 4 qPCR VII Kit
Kit Version: 4.0



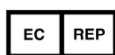
300107 (50 Reactions)
300108 (100 Reactions)



Store at -25°C to -15°C



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For use on Bio-Rad CFX96 and abCyclerQ only.

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 Orthomyxoviridae 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 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. 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 abTES™ FLU 4 qPCR VII 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. The abTES™ FLU 4 qPCR VII Kit enables detection of the human housekeeping gene, GAPDH as an **Internal Control (IC)** to identify possible PCR inhibitions and sampling problems.

3. Storage Conditions

The components of abTES™ FLU 4 qPCR VII 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 | 300107 (50 rxns) | 300108 (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 and abCyclerQ. You may need to adjust these conditions for other real-time platforms. **FAM** (FluA), **Texas Red** (FluB), **HEX** (swH1N1), **Cy5** (huH3N2) and **Quasar 705/Alexa Fluor 680** (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. Performance Characteristics

All reactions for performance characteristics were performed using the abTES™ FLU 4 qPCR I Kit on the Bio-Rad CFX96 and abCyclerQ. The reaction composition, cycling condition and primer/probe design for the swH1N1, FluA, FluB and huH3N2 targets of the abTES™ FLU 4 qPCR I Kit are identical to this kit. The data generated is therefore considered a good representative of the performance of this kit.

8.1. Analytical Sensitivity

Analytical sensitivity (limit of detection) is defined as 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 | Detecting channel | Analytical Sensitivity (95% confidence) | |
|--------|-------------------|-----------------------------------------|-----------------|
| | | CFX96 | abCyclerQ |
| swH1N1 | HEX | 0.22 copies/µl | 0.55 copies/µl |
| FluA | FAM | 0.41 copies/µl | 1.03 copies/µl |
| FluB | Texas Red | 5.92 copies/µl | 14.80 copies/µl |
| huH3N2 | Cy5 | 0.29 copies/µl | 0.73 copies/µl |

8.2. 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% |

abTES™ FLU 4 qPCR VII Kit

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 | abTES (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 | abTES (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 | abTES (n=218) | | Sensitivity/ Specificity |
|------------------|---------------|---------------|--------------------------|
| | FluB Positive | FluB Negative | |
| FluB Positive | 50 | 0 | 100% sensitivity |
| FluB Negative | 0 | 168 | 100% specificity |
| Total | 50 | 168 | Total |

Diagnostic sensitivity and specificity of huH3N2:

| Reference Method | abTES (n=218) | | Sensitivity/ Specificity |
|------------------|-----------------|-----------------|--------------------------|
| | huH3N2 Positive | huH3N2 Negative | |
| huH3N2 Positive | 63 | 0 | 100% sensitivity |
| huH3N2 Negative | 0 | 155 | 100% specificity |
| Total | 63 | 155 | |

9. 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 when the FAM, HEX, Texas Red and Cy5 channels are negative and the Quasar 705/Alexa Fluor 680 Internal Control channel is positive.

| Result | FluA (FAM) | swH1N1 (HEX) | FluB (TxR) | huH3N2 (Cy5) | IC (Q705) |
|------------------------|------------|--------------|------------|--------------|-----------|
| FluA positive | + | - | - | - | +/- |
| swH1N1 positive | + | + | - | - | +/- |
| FluB positive | - | - | + | - | +/- |
| huH3N2 positive | + | - | - | + | +/- |
| FluA and FluB negative | - | - | - | - | + |
| Indeterminate | - | - | - | - | - |

10. 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.
- Check proper volume of the reagent added during the PCR setup.









10.2. 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.

10.3. 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.

11. Explanation of Symbols

| | |
|--------------------------------------------------------------------------------------|-----------------------------------------------------|
|  | <i>In vitro</i> diagnostic medical device |
|  | Catalogue number |
|  | Store at -25°C to -15°C |
|  | Manufacturer |
|  | Authorized representative in the European community |
|  | 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/>