LiliF™ AIV H5/H7/H9 Real-time RT-PCR Kit

RUO Research Use Only REF IP15002 Σ 50 -20℃

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Belief & Relief, LiliF Diagnostics MDx Kit
Instruction For Use
English (영문, 英語)

BACKGROUND INFORMATION

Avian influenza virus (AIV) is a disease caused by type A influenza virus, a member of the Orthomyxoviridae family. Influenza A viruses are responsible for major disease problems in birds, as well as in mammals including humans. Infection of domestic poultry by AIV viruses typically produces syndromes ranging from mild, localized infection such as respiratory disease and drops in egg production to severe, systemic disease with near 100% mortality. Aquatic avian species are thought to be reservoirs of AIV in nature.

Influenza A viruses are negative-sense, single-stranded, segmented RNA viruses. The several subtypes are labeled according to an H number (for the type of hemagglutinin) and an N number (for the type of neuraminidase). There are 18 different H antigens (H1 to H18) and 11 different N antigens (N1 to N11). H17 was isolated from fruit bats in 2012. H18N11 was discovered in a Peruvian bat in 2013.

Some strains of the H5, H7 and H9 AIV cause systemic infection with a high mortality rate in poultry species and are thus categorized as highly pathogenic avian influenza (HPAI) viruses.

LiliF™ AIV H5/H7/H9 Real-time RT-PCR Kit is able to detect directly and specifically Avian influenza virus highly pathogenic serotype (H5, H7 and H9) by real-time RT-PCR method on the basis of a genetic database, so it can diagnose very sensitive, fast and accurately. It is designed to detect AIV H type 5, 7 and 9 with high sensitivity and high specificity. Primer and probes of the Kit are based on current sequence alignments of all Avian influenza virus subtypes (1 – 12), allowing the RNA detection (typing). It can determine the infecting serotype and accurately and sensitivity detect multiple pathogens at one time using the real-time RT-PCR (quantitative) method, and take only 2 hours for detection. Fast and sensitive detection of pathogen enables patients to get appropriate treatment and prevent the rapid spreading of disease by separating patients immediately.

PRINCIPLES

- This product is a qualitative Real-time PCR testing product with 5' nuclease assay technology and CLP™ technology which provided flexibility in Tm (melting temperature) of primer design for optimization of reaction condition, and maximizes PCR specificity and sensitivity through the control of non-specific priming.
- The assay is a real-time RT-PCR that discriminates AIV in one reaction. The assay is composed of two principal steps: (1) nucleic acid extraction from specimens, and (2) amplification of the target extracted nucleic acid fragment using fluorescent probe and specific primers pair.

INTENDED USE

For Research Use Only, Not for use in diagnostic procedures.

This kit is developed, designed, and sold for research purpose only. It is not intended to be used for human or animal diagnosis of diseases. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations.

This product is research reagent of infectious disease for professional use to restrict the public use for animal diseases.

KIT CONTENTS

No.	Contents	Composition
1	2X qRT-PCR Master Mix Solution	< 0.01% Hot start Taq DNA Polymerase < 0.01% Reverse Transcriptase < 0.01% dATP, dTTP, dGTP, dCTP
2	AIV H5/H7/H9 Detection Solution	<0.005% Primer/probe
3	Positive Control	< 0.001% Non-infectious plasmid DNA(microbial) containing AIV H5/H7/H9 primer binding sequences
4	DNase/RNase Free Water	No template control < DNase/RNase Free Water

DESCRIPTION

1. 2X qRT-PCR Master Mix Solution : Colorless and transparent liquid in microtube
2. AIV H5/H7/H9 Detection Solution : Colorless (pale-pink colored) and transparent liquid in dark brown colored amber tube
3. Positive Control : Colorless and transparent liquid
4. DNase/RNase Free Water : Colorless and transparent liquid

REAGENTS & CONSUMABLES TO BE SUPPLIED BY USER

- Real-time PCR Instrument
- Pipettes & Sterile pipette tip (with filter)
- Disposable gloves
- Centrifuge for micro-centrifuge tubes
- Nucleic acid extraction kit
- Table top centrifuge
- Vortex mixer
- Passive reference dye (Optional)

NOTICE BEFORE USE

※ Precautions before Testing

- All procedures must be done on a clean bench that should be cleaned with 70% alcohol or 10% household bleach (Na-hypochlorite) after use.
- The experimenter should wear a lab coat gloves, masks, etc., and to always be careful.
- The specimen used should be kept separate. If discarded, it is considered to be a biological hazardous substance after high-pressure sterilization and discarded.

※ Preparation, Preservation and Transportation of Specimen

1) Specimen type
   - NP swab, throat swab, nasal wash, nasal aspirate, oropharyngeal swab, cloacal swab, sputum.
   - Keep samples below - 20℃ before use, unless you have prepared them right before use.
2) How to deliver and store the specimen
   - Store the specimen at 4℃ with ice in the ice box and deliver it quickly to the test centers
   - Keep the specimen away from the ice to avoid freezing in the case of liquid sample
   - Store -20℃ or -70 ℃ when the sample can not be delivered immediately
   - Store -20℃ for a week
3) Cautions of extraction and delivery of specimen
   - Wear the protection equipment before collecting the specimens
   - Deliver the specimen immediately under 4℃ with a written request
   - Personal protection equipment : N-95 mask, gloves, safety goggles, bumper cap, overshoes
4) Inappropriate specimen : The following aspects should be avoided to test
   - Samples against the standard of epidemiology or clinical diagnosis
   - Samples with inappropriate delivery temperature or incorrect container
   - Samples which is spoiled from the container

※ Preparation before Testing

1. Preparation of Reagents
   1) 2X qRT-PCR Master Mix Solution, AIV H5/H7/H9 Detection solution
      - Before starting the test, take out the necessary quantity and use it.
      - Do not leave it at room temperature more than 1 hour. Repeated freezing and thawing may affect the performance of the product.
   2) DNase/RNase Free Water / Positive Control
      - Leave it at 4 ℃ or room temperature for thawing. Do not leave it at room temperature more than 1 hour.
2. Nucleic Acid (DNA or RNA) Extraction

   Using the nucleic acid extraction kit as general-purpose DNA or RNA extraction kit, to isolate the nucleic acids from the specimen. Follow the instruction for use of each extraction kit. The extraction method described below is an example of how to use our extraction kit. e.g.) Patho Gene Spin™ DNA/RNA Extraction Kit (iNtRON. Cat. No. 17154)

1) Transfer 150 µl specimen in the 1.5 ml micro-centrifuge tube.
   Note : If the sample volume is less than 150 µl, fill up PBS by 150 µl.
2) Add 300 µl of Lysis buffer and vortex 15 sec.
3) Incubate at room temperature (15-25℃) for 10 min.
4) Add 300 µl of Binding Buffer, and completely mix well by gently vortexing.
5) Transfer 750 µl of lysate in the column and centrifuge at 13,000rpm for 1min.
6) Discard solution in collection tube and place the column back in the same 2ml collection tube.
7) Add 500 µl of Washing Buffer A to column and centrifuge for 1min at 13,000rpm.
8) Discard solution in collection tube and place the spin column back in the same 2 ml collection tube.
9) Add 500 µl of Washing Buffer B to the column and centrifuge for 1min at 13,000rpm.
10) Discard solution in collection tube and place the spin column back in the same 2ml collection tube. Centrifuge for 1min at 13,000rpm again.
    Note : It is important to dry the membrane because residual ethanol may interfere with downstream reactions.
11) Place the column in a RNase-free 1.5 ml micro-centrifuge tube (not provided)
12) Add 30-50 µl of Elution Buffer directly onto the membrane. Incubate at RT for 1min, and then centrifuge for 1min at 13,000rpm.

STORAGE CONDITION & SHELF-LIFE

No.	Reagents	Storage conditions	Shelf-Life
1	2X qRT-PCR Master Mix Solution	-25℃~-15℃	Within 6 months after opening, within expiry date of the kit
2	AIV H5/H7/H9 Detection Solution	-25℃~-15℃	Within 6 months after opening, within expiry date of the kit
3	Positive Control	-25℃~-15℃	Within 6 months after opening, within expiry date of the kit
4	DNase/RNase Free Water	-25℃~-15℃	Within 6 months after opening, within expiry date of the kit

LiliF Diagnostics

※ Testing Protocols

1. Prepare the tube of AIV Real-time Detection Master Mix as +2 quantity of the number of samples.

⚠ An appropriate number of tubes means the combination of two tubes in the number of samples, which includes a positive control and a negative control. In case of real time PCR, the fluorescent signal is passed through the transparent cap of the PCR tube. Be sure not to label the cap and be able to identify it by a separate way.

AIV Real-time Detection Master Mix	
2X qRT-PCR Master Mix Solution	10 µl
AIV H5/H7/H9 Detection Solution	5 µl
Total volume	15 µl

2. Add 5 **µl** of extracted nucleic acid, Positive Control or NTC (no template control; DNase/RNase Free Water) to each prepared premix and close the tube cap.

⚠ For the negative control, use 5µl DNase / RNase Free Water instead of the genomic sample and 5µl of Positive Control DNA sample included in the kit for positive control.

⚠ Real-time PCR is highly sensitive and can easily contaminate negative control samples. Therefore, it is recommended to use an aerosol barrier tip (filter tip) or a pipette only for positive control to prevent contamination.

3. Mix thoroughly by vortexing then spin down the reaction tube by centrifuge to remove bubble of mixture and liquid on the tube walls.

⚠ Be careful not to shuffle the tubes in this procedure because real time PCR does not label the tubes separately.

4. Perform the PCR reaction according to the program described below.

Step	Cycle	Temp	Time
Reverse Transcription	1	45 ℃	30 min.
		95 ℃	10 min.
PCR and Signal Detection	40	95 ℃	15 sec.
		58 ℃	60 sec.

Channel setting	
H type 5	FAM
H type 7	HEX
H type 9	Cy5

☛ Gray shaded area means signal detection step

ANALYSIS AND INTERPRETATION OF RESULTS

※Interpretation of result

1. It is recommended to refer to the manual of the relevant instrument because the analysis method differs according to the Real-time PCR machine used.
2. It is advisable to judge the negative control first and proceed with the result judgment on the sample when it appears as a negative judgment.
3. Using a positive control that is provided, it can verify the validity of the PCR reaction itself.
4. The criteria for interpreting the results are as follows.
   - Manual Baseline : automatic baseline
   - Threshold setting : The threshold value of the real-time PCR is usually set at 5 ~ 10% of the Rn value of the positive control, which may vary depending on the equipment. We recommend that you refer to the following table [analytical parameters] for analysis.
   - Ct cut off value : It is recommended to exclude values after cut-off value based on the Ct value corresponding to the threshold value selected through the following table.

[Table] Analytical parameters

Baseline Setting	Manual baseline	Threshold	Ct Cutoff Value
Auto	3~15	Auto	Drop after 36 cycle

[Table] The Criteria of Data interpretation

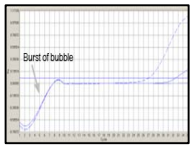
Items	FAM	HEX	Cy5
Positive Control	18 ~ 22	22 ~ 25	22 ~ 25
Negative Control	≥ 36	≥ 36	≥ 36
Sample (Positive case)	< 36	< 36	< 36
Sample (Negative case)	≥ 36	≥ 36	≥ 36

5. Quality Control
   This product contains a positive control in the product. Therefore, in order for the user to judge whether the performance of this product is working properly, please check whether the positive result and the negative reference solution react with each other and whether the result is normal. If the proper storage environment and unusual results within the life of the product are obtained, the manufacturer may be asked to exchange the product.
6. Example Results

No.	Samples	H5 (FAM)	H7 (HEX)	H9 (Cy5)	Interpretation
1	Positive control	+	+	+	valid
2	Negative control	-	-	-	valid
3	Test 1	+	-	-	positive (H5)
4	Test 2	-	+	-	positive (H7)
5	Test 3	-	-	+	positive (H9)
6	Positive Control	-	-	-	retest
7	Negative Control	+	-	-	contamination

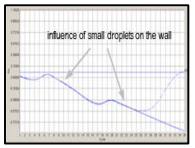
※ Interpretation of abnormal results

[Bubble formation]



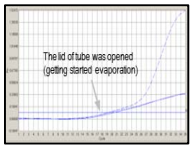
There is large bubble in lower part of tube. A sharp increase of signal intensity is originated from a burst of bubbles on the way of reaction.

[Droplet on the wall]



There are some small droplets on the wall of a tube. The increase of signal intensity resulted from lots of small droplets but much small droplets.

[Sealing error]



Completely close the lid of the tube is if you do not. As the reaction proceeds baseline results appear to rise slightly.

CAUTIONS

1. All procedures must be done on a clean bench and clean bench should be wiped clean with alcohol after use.
2. Always wear protective gear during handling chemical materials and the test should be handled by professionally trained person.
3. The every specimen may have the risk of infection and unknown diseases, so be careful when handling them to prevent infection by users and indirect contacts.
4. Do not mix reagents from different lots of this product.
5. Carefully treat the reagents and specimens of this product to prevent the aerosol from splashing when opening the lid of the container and prevent reagents and specimens from splashing by wearing a mask.
6. Be careful not to spill the aerosol when you open the container lid by carefully handling the reagents and samples of this product. In addition, wear a mask to prevent reagents and specimens from splashing on your mouth.
7. During the handling of this product and specimens, do not place any instruments that may hurt you, such as needles or knives, and avoid safety accidents by not using such instruments.
8. If you want to dispose of suspected specimens, contaminated test materials and instruments, disinfect them using high pressure steam sterilization or disinfection. If you want to disinfect, treat with 70% ethanol, 10% bleach solution for 10 ~ 30 minutes .
9. Because the optical tube of Real time PCR which is used according to each equipment is different, this product can be supplied in a customized form quickly without stock. Therefore, before ordering, please check the compatible profile of the tube and the model of the equipment you are using and contact us.
10. All reagents contained in the kit should be stored at -20℃.
11. Be aware of contamination or direct contact from test specimens.
12. For more accurate testing, we recommend using samples taken within 5 days after symptoms.
13. To prevent contamination, we recommend that you observe the following :
    - It is advisable to separate extraction space and PCR space so that they do not overlap.
    - Centrifuge, test bench and pipette should be periodically cleaned with Bleach solution (10% house-hold bleach) to prevent the entry of unknown contaminants.
    - Treat the reaction solution in the order of negative control (NTC) → sample → positive control during PCR reaction.

PACKINPACKAGING INFORMATION

No	Contents	50 Test / kit
1	2X qRT-PCR Master Mix Solution	280 µl x 2 tubes
2	AIV H5/H7/H9 Detection Solution	140 µl x 2 tubes
3	Positive Control	25 µl x 3 tubes
4	DNase/RNase Free Water	1 ml x 1 tube

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EXPLANATION OF SYMBOLS

Manufactured by | Attention | Consult Instructions For Use

Manufacturing date | Expire date | Keep away from sunlight

Sufficient for tests | Do not reuse | Storage temperature limitation

Batch number | In vitro diagnostic | Product number

LOT | IVD | REF

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