Result Analysis and Judgments

Channel	Test Results	Result Judgment
FAM	Ct Value≤40	AIV H5 subtype is positive
	Ct Value＞40	It needs to be tested again, and the re-test results are still within this range or met positive criteria, it is determined to be positive for AIV H5 subtype, otherwise was negative.
	No Ct value	AIV H5 subtype is negative
HEX	Ct Value≤40	AIV N1 subtype is positive
	Ct Value＞40	It needs to be tested again, and the re-test results are still within this range or met positive criteria, it is determined to be positive for AIV N1 subtype, otherwise was negative.
	No Ct value	AIV N1 subtype is negative

Note

1. The whole process should be carried out in different area. Area I for preparing amplification reagent, area II for processing of tested samples, and area III for PCR amplification detection. All the articles in each district are for special use which cannot allow to be exchanged for avoiding pollution. The workbench should be cleaned immediately after the completion of each experiment.
2. Please handling samples in the biosafety cabinet, to ensure the safety of operators and avoid environmental pollution. Place harmful samples and reagents properly. Discard the waste in special containers. Wipe the table, centrifuge, and equipment frequently with 1.0% sodium hypochlorite or 70% ethanol. The laboratory and the ultra-clean workbench need UV-treated periodically and after each experiment.
3. Both the kit and nucleic acid products are all stored at -20℃. They should be fully melted, gently mixed and briefly centrifuged before use. Use disposable gloves without fluorescent substances, disposable special centrifuge tubes, etc.
4. After amplification, please take out the reaction tube, seal it in the special plastic bag, put it in the designated place, and wait for unified treatment.
5. The kit should be used within the shelf life. Different batches should not be used together.

Information of Manufacturer

Manufacturer: Hangzhou Bioer Technology Co., Ltd.
Address: 1192 BinAn Rd, Binjiang District 310053 Hangzhou,
PEOPLE'S REPUBLIC OF CHINA
Website: www.bioer.com.cn
Tel: +86-571-87774567
FAX: +86-571-87774565

Avian Influenza Virus H5N1 Subtype Nucleic Acid Detection Kit (Fluorescence PCR)

TECHNICAL SUPPORT:

For technical support, please dial phone number ： 0086-571-87774567-5287 or 5297, or fax to 0086-571-87774553
Email to reagent@bioer.com.cn.
Website: www.bioer.com.cn

Intended Use

This kit can be used for qualitative testing of avian influenza virus（AIV） H5N1 subtype in tissue, blood, or throat swab of suspected infection or other animals that require the diagnosis or differential diagnosis of avian influenza virus H5N1 subtype infection (chicken, duck, goose, etc.). The test results of this kit are for clinical reference only and should not be used as the sole basis for diagnosis or exclusion.

Principle

The kit selects specific conserved regions of AIV H5N1 subtype to design primers and probes. The detection system uses two different fluorophores to independently detect AIV H5 subtype target gene (FAM channel) and AIV N1 subtype target gene (HEX channel).

Ingredients

Ingredient	BSL58S1	BSL58M1
size	24T	48T
RT-PCR Buffer	300 μL	600 μL
Enzyme Mix	31.2 μL	62.4 μL
H5N1 Primer/Probe	148.8 μL	297.6 μL
Negative Control	200 μL	200 μL
Positive Control	200 μL	200 μL

Storage and period of validity

1. The kit need to be transported under freezing conditions.
2. The kit should be stored at -25℃~-15℃ away from light, and avoid repeated freeze-thaw more than 5 times.
3. The kit can be stored for up to 12 months if all components are kept in the manner above. (Please use the kit in the period of validity).

Applied instruments

It can be used Line-Gene series fluorescence quantitative PCR detection system from Bioer or similar instruments from other manufacturers.

Sample request

The collected samples should be placed in sterile and non-polluting homogenous bags to avoid cross-contamination of the experiment. If the samples are not extracted immediately, it should be stored at -20℃. The nucleic acid detected by the kit should be extracted from samples of tissue, blood, or throat swab of chicken, duck, goose and poultry animal.

Using of the kit

1. Nucleic acid extraction

Samples can be extracted using the Simply P Virus DNA/RNA Extraction Kit (Cat No.: BSC67), Biospin Virus DNA/RNA Extraction Kit (Cat No.: BSC77) and MagaBio plus Virus DNA/RNA

Purification Kit III (Cat No.: BSC86) from Bioer.

2. Amplification reagent preparation (PCR preparation area)

Thaw out the reagents at room temperature. Before preparing PCR reagents, mix gently and centrifuge briefly all reagents. Make PCR reagents according to the quantity of samples and controls as below (N tests add an extra blank control):

Reagent	RT-PCR Buffer	Enzyme mixture	H5N1 Primer / Probe
Dosage / test	12.5 μL	1.3 μL	6.2 μL
Dosage	(N+1) ×12.5 μL	(N+1) ×1.3 μL	(N+1) ×6.2 μL

After mixing PCR reagents above, distribute 20 μL into every PCR tube, and then transfer to sample disposal area.

3. Adding sample (Sample treatment area)

Add 5 μL extracted positive control, negative control and unknown samples into every PCR tube. Tighten the tube cover, remove bubbles by centrifuge, and then conduct PCR reaction.

4. PCR reaction (PCR amplification area)

The PCR tube was put into the instrument. The negative control, positive control and unknown samples were placed set according to in the corresponding order. Select the **FAM channel (AIV H5 subtype) and HEX channel (AIV N1 subtype)** of instrument for fluorescent signal collection. Set reaction procedure as following:

50℃ 5 min
95℃ 1 min
95℃ 5 sec
60℃ 20 sec
} 45 Cycles

Instrument set fluorescent signals detecting at 60℃, reagent volume is 25 μL.

Quality Control Standards

	FAM	HEX	Interpretation of Test Results
Positive Control	Ct value ≤ 35	Ct value ≤ 35	All conditions are met in the same experiment, indicating that the experiment is valid, otherwise it is invalid.
Negative Control	No Ct value	No Ct value	