

CARE FOR LIFE WITH SCIENCE AND TECHNOLOGY

## Avian Influenza Virus Nucleic Acid Detection Solution

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## **Product information about Avian Influenza Virus Nucleic Acid Detection**

#### Introduction

Avian influenza virus (AIV) is one of the influenza A viruses and is classified by the World Organisation for Animal Health (OIE) as a category A virulent infectious disease. It can be divided into 16 H subtypes (H1 to H16) and 9 N subtypes (N1 to N9) depending on the antigenicity of its outer membrane haemagglutinin (H)/ and neuraminidase (N) proteins. Among the avian influenza viruses, H5N1, H7N7 and H9N2 can be transmitted to humans, with H5N1 being highly pathogenic and having a high mortality rate. Avian influenza has caused serious stagnation in the sale of poultry products and heavy losses to farmers, causing great economic losses to the poultry farming industry.

#### **Bioer AIV Detection Method**

The RT-PCR technique has the advantage of high specificity and sensitivity, and real-time fluorescence RT-PCR is the recommended method for nucleic acid detection of pathogens in the laboratory. The detection of avian influenza viruses is not only used for disease diagnosis, epidemiological investigation and pathogenesis research, but also for the diagnosis of multiple subtypes and differentiation of different subtypes. Based on our mature molecular diagnostic platform and technology, we have developed avian influenza virus nucleic acid test reagents to meet the needs of different customer groups.

Product Name	Catalogue Number	Packing Size (Tests/Kit)	Test objects	Storage Condition
Avian influenza virus H5 subtype nucleic acid detection kit (Fluorescence PCR)	BSL49S1/BSL49M1	24T/48T	Avian influenza virus H5 subtype	-20°C
Avian influenza virus H7 subtype nucleic acid detection kit (Fluorescence PCR)	BSL50S1/BSL50M1	24T/48T	Avian influenza virus H7 subtype	-20°C
Avian influenza virus H9 subtype nucleic acid detection kit (Fluorescence PCR)	BSL51S1/BSL51M1	24T/48T	Avian influenza virus H5 subtype	-20°C
Avian influenza virus nucleic acid detection kit (Fluorescence PCR)	BSL52S1/BSL52M1	24T/48T	Avian influenza virus	-20°C
Avian influenza virus H5/H7/H9 subtype nucleic acid detection kit (Fluorescence PCR)	BSL53S1/BSL53M1	24T/48T	ldentification of avian influenza virus subtypes H5, H7 and H9	-20°C

#### **Reagent kit characteristics**

- Sample Types: Animal plasma, serum, swabs, tissue and virus cultures.
- High Sensitivity: Detection limits up to 500copies/mL.
- High Specificity: Distinguishes between H5/H7/H9 subtypes and maintains good negative results for all influenza B tests.
- Wide Applicability: It can be used with all kinds of fluorescence PCR instruments on the market, especially with the fluorescence PCR instruments from BIOER.
- Simple Operatation and Short Time Consuming: Pre-mixed kit with few components for easy handling; PCR reaction can be completed in just 1h.



#### Application examples (or experimental results)

#### **Case 1: specificity test for Avian influenza virus nucleic acid test kit**

H1N1, H3N2, H5N1, H7N9, H9N2, B-V and B-Y positive samples were tested by the kits respectively, The results show that: avian influenza virus nucleic acid detection kit can detect H1N1, H3N2, H5N1, H7N9, H9N2 samples, the rest of the samples are negative; avian influenza virus H5 subtype can only detect H5N1 samples, the rest of the samples are negative; avian influenza virus H7 subtype can only detect H7N9 samples, the rest of the samples are negative; avian influenza virus H7 subtype can only detect H7N9 samples, the rest of the samples are negative; avian influenza virus H7 subtype can only detect H7N9 samples are negative; indicating that the above kits do not cross with other viruses and have good specificity, the results are summarized as follows:

	general	H5 subtype	H7 subtype	H9 subtype
H1N1	27.56	NoCt	NoCt	NoCt
H3N2	28.17	NoCt	NoCt	NoCt
H5N1	17.53	17.85	NoCt	NoCt
H7N9	27.47	NoCt	30.97	NoCt
H9N2	24.18	NoCt	NoCt	23.6
B-V	NoCt	NoCt	NoCt	NoCt
B-Y	NoCt	NoCt	NoCt	NoCt
Newcastle disease virus	NoCt	NoCt	NoCt	NoCt
duck plague virus	NoCt	NoCt	NoCt	NoCt
Chicken Infectious bursal virus	NoCt	NoCt	NoCt	NoCt
Chicken Infectious Bronchitis Virus	NoCt	NoCt	NoCt	NoCt



Avian influenza virus



Avian influenza virus H7 subtype



Avian influenza virus H5 subtype



Avian influenza virus H9 subtype

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Positive samples of H5N1, H7N9 and H9N2 strains of avian influenza were tested 10 times with the corresponding reagents (H5N1 with H5 subtype nucleic acid detection kit, H7N9 with H7 subtype nucleic acid detection kit, H9N2 with H9 subtype nucleic acid detection kit, with H5N1, H7N9 and H9N2 samples) and the Ct coefficient of variation (CV) for each subtype was found to be less than 1%, indicating good precision of the kits.

#### 2.1 H5 subtype nucleic acid detection kit





#### 2.2 H7 subtype nucleic acid detection kit



N	117110
NO.	HIN9sample
1	32.68
2	32.37
3	32.45
4	32.55
5	32.49
6	32.3
7	32.54
8	32.55
9	32.61
10	32.75
CV	0.42%

#### 2.3 H9 subtype nucleic acid detection kit



#### 2.4 Avian influenza virus H5/H7/H9 subtype nucleic acid detection kit



No.	FAM (H5)	HEX (H7)	ROX (H9)
1	30.61	30.80	31.95
2	30.57	30.57	31.78
3	30.38	30.40	31.66
4	30.34	30.42	31.47
5	30.08	30.13	31.22
6	30.01	30.06	31.3
7	29.99	30.16	31.17
8	30.05	30.17	31.13
9	30.53	30.62	31.78
10	30.49	30.62	31.84
CV	0.82%	0.84%	0.98%

#### I Case 3

Each of the above kits was placed at 37°C for 7 days and tested for different concentrations of positive samples of avian influenza virus. The test results were not significantly different from those of the control reagents stored at normal storage temperature (-20°C), indicating that the stability of the above kits was good. The results of the experiments are summarised as follows:

3.1 results of Avian influenza virus H5 subtype nucleic acid detection kit at 37°C is accelerated 7 days:



	-20°C	37°C
sample 1	25.22	25.19
sample 2	28.64	28.65
sample 3	31.79	32.06
sample 4	34.40	34.59

3.2 results of Avian influenza virus H7 subtype nucleic acid detection kit at 37°C is accelerated 7 days:



	-20°C	37°C
sample 1	25.48	26.06
sample 2	29.25	29.65
sample 3	33.11	32.78
sample 4	36.50	37.44

-20°C

25.21

28.54

sample 1

sample 2

sample 3

sample 4

37°C

25.51

28.91

31.76

35.11

3.3 results of Avian influenza virus H9 subtype nucleic acid detection kit at 37°C is accelerated 7 days:

3.4 results of Avian influenza virus nucleic acid detection kit at 37°C is accelerated 7 days:



18 20 22 24 26 28 20 22 34 26 38 40 Cycles

Fluorescence

sample 3	31.83	32.10
sample 4	34.71	35.33
	-20°C	37°C
sample 1	24.39	24.54
sample 2	27.93	27.88

31.73

34.72

#### 3.5 results of Avian influenza virus H5/H7/H9 subtype nucleic acid detection kit freeze-thaw 7 times or accelerated 7 days at 37°C:



		-20°C		freeze-thaw 7			accelerated 7 days at 37°C		
sample	FAM	HEX	ROX	FAM	HEX	ROX	FAM	HEX	ROX
1	25.92	25.95	26.73	26.12	26.26	26.87	26.10	25.97	26.79
2	28.99	29.19	29.95	28.95	29.12	29.96	29.31	29.37	30.18
3	32.83	32.93	34.05	32.63	33.08	34.22	32.92	33.15	33.85
4	36.66	36.40	37.15	37.01	37.16	37.65	36.78	36.51	37.82

Five positive samples of avian influenza virus were tested with the avian influenza virus nucleic acid test kit and other brands of the kit in market, it showed comparable results between the two kits, which are summarized below



The graph a shows the amplification curve of the BIOER kit (reaction time 55 min) and the graph b shows the amplification curve of other brand kit (reaction time 90 min).

## Avian Influenza Virus Lyophilized Nucleic Acid Detection Kit (Fluorescence PCR)

#### Introduction

Avian influenza (AI) viruses, commonly known as bird flu, are members of the family orthmyxoviridae, Type A which is subdivided into categories (strains) depending on the outer proteins H (Haemagglutinin) and N (Neuraminidase). There are many types of avian influenza, which are all caused by various strains of type A influenza virus (e.g., H5N1, H7N3, H9N2). Avian influenza A(H5N1) illness is caused by the avian influenza A(H5N1) virus. The disease spreads by direct contact or through contaminated faeces and bodily fluids. New AI virus strains are created frequently which means that there is a constant risk that one of the new strains may spread easily among people. Avian Influenza continues to be a threat to the worldwide poultry industry. An effective control strategy for avian influenza in domesticated poultry is therefore an essential element in the protection of both bird and human populations. A laboratory test is required to diagnose Avian influenza (AI) viruses, Real-Time PCR technology has the advantages of high specificity and sensitivity, so it is recommended for laboratory detection. Based on its mature molecular diagnosis platform and technical reserves, Bioer Technology has developed related products of avian influenza virus nucleic acid detection reagents.

#### **Bioer AIV Detection Method**

RT-PCR technology has the advantages of high specificity and sensitivity, and the real-time fluorescent RT-PCR method is recommended for laboratory pathogen nucleic acid detection. The detection of AIV is not only used for disease diagnosis, epidemiological investigation, and pathogenesis research, but also can diagnose multiple subtypes and distinguish different subtypes. Based on its own mature molecular diagnosis platform and technical reserves, Bioer has developed related products of AIV nucleic acid detection reagents according to the needs of different customer.



Product Name	Catalogue Number	Packing Size (Tests/Kit)	Storage Condition
Avian Influenza Virus Lyophilized Nucleic Acid Detection Kit (Fluorescence PCR)	BSL78S1	48T	Room Temperature

- Sample Type: Animal plasma, serum, swab, tissue, and virus culture can be selected.
- High Sensitivity: The detection limit can reach 200 copies/ml.
- High Specificity: It can effectively detect Avian Influenza virus, and no cross-reaction with Influenza B Virus, Newcastle Disease Virus, Infectious Bursal Disease Virus, Avian Infectious Bronchitis Virus, Avian Infectious Laryngeal Tracheitis Virus, Avian Maorik's Disease Virus, Chicken Mycoplasma, Salmonella, Chicken Genomic DNA.
- Wide Applicability: It can be compatible with various fluorescent quantitative PCR instruments on the market, especially Bioer Real-Time PCR instruments.
- **Good Stability:** The reagent kit adopts the form of lyophillization. After lyophillization, the active substances (such as enzymes) in the product will be protected at ambient temperature, and can be transported at room temperature, improving and extending the stability of the reagent, greatly reducing the problems of reagent performance degradation or failure caused by temperature changes.
- Simple Operation and Short Time Consumption: The reagent kit does not need to be prepared, and after re-dissolution, it only needs to be packaged in one step to add samples for testing, and the test results can be obtained within 1 hour.

#### **Application Cases**

#### Case 1

The standard curve was drawn after using the kit to detect the positive samples of avian influenza virus. The correlation coefficient of Ct value of the target gene of avian influenza virus was 1.000, indicating that the kit had a good linear relationship and high PCR efficiency. The results are shown in the following figure:



Figure 1 Standard curve of avian influenza virus detection kit



Figure 2 Amplification curve of avian influenza virus detection kit

H1N1, H3N2, H5N1, H7N9, H9N2, Influenza B Virus, Newcastle Disease Virus, Infectious Bursal Disease Virus, Chicken Infectious Bronchitis Virus, Avian Infectious Laryngeal Tracheitis Virus, Chicken Maorik's Disease Virus, Chicken Mycoplasma, Salmonella and Chicken genomic DNA were detected by using the kit. The results showed that: Except for H5N1, H7N9 and H9N2, the other samples were negative, indicating that the kit did not cross with other viruses and had good specificity.



Figure 3. Amplification curve of the cross-validation experiment of the reagent kit

Sample	Results
H5N1	+
H7N9	+
H9N2	+
H1N1	+
H3N2	+
Influenza B Virus	-
Newcastle Disease Virus	-
Infectious Bursal Disease Virus	-
Chicken Infectious Bronchitis Virus	-
Avian Infectious Laryngeal Tracheitis Virus	-
Chicken Maorik's Disease Virus	-
Chicken Mycoplasma	-
Salmonella	-
Chicken genomic DNA	-

Table 1. Summary of cross-validation results for reagent kits Note: "+" represents a positive result, and "-" represents a negative result.

## Avian Influenza Virus H5/H7/H9 Subtype Lyophilized Nucleic Acid Detection Kit (Fluorescence PCR)

#### Introduction

Avian Influenza Virus (AIV) is a kind of influenza A virus, which is listed as a severe infectious disease of type A by the World Organization for Animal Health (Founded as OIE). According to the antigenicity of the outer membrane hemagglutinin (H) and neuraminidase (N) proteins, it can be divided into 16 H subtypes (H1-H16) and 9 N subtypes (N1-N9). Among avian influenza viruses, highly pathogenic avian influenza is caused by the H5 and H7 subtypes of influenza A virus, which are highly harmful and have a high mortality rate. Additionally, H5N1, H7N9, and H9N2 can be transmitted to humans. Therefore, if not detected timely, it may result in in heavy losses for farmers and causing significant economic losses to the poultry breeding industry. RT PCR technology has the advantages of specificity and high sensitivity, and real-time PCR method is recommended for laboratory pathogen nucleic acid detection. The detection of avian influenza virus is not only used for disease diagnosis, epidemiological investigation, and pathogenesis research, but also for diagnosing multiple subtypes and distinguishing different subtypes. Bioer Technology has developed products related to nucleic acid testing reagents for avian influenza virus based on its mature molecular diagnostic platform and technological reserves.

#### **Bioer AIV Detection Method**

RT-PCR technology has the advantages of high specificity and sensitivity, and the real-time fluorescent RT-PCR method is recommended for laboratory pathogen nucleic acid detection. The detection of AIV is not only used for disease diagnosis, epidemiological investigation, and pathogenesis research, but also can diagnose multiple subtypes and distinguish different subtypes. Based on its own mature molecular diagnosis platform and technical reserves, Bioer has developed related products of AIV nucleic acid detection reagents according to the needs of different customer.

Product Name	Catalogue Number	Packing Size (Tests/Kit)	Detect Target	Storage Condition
Avian Influenza Virus H5/H7/H9 Subtype Lyophilized Nucleic Acid Detection Kit (Fluorescence PCR)	BSL76S1	48T	AIV H5&N7&N9 subtype	-20°C

- Sample Type: Animal plasma, serum, swab, tissue, and virus culture can be selected.
- High Sensitivity: The detection limit can reach 200copies/ml.
- High Specificity: It can effectively distinguish H5/H7/H9 subtypes, and has no cross reaction with H1N1, H3N2, influenza B
  virus, Newcastle disease virus, infectious bursa of fabricius disease virus, avian infectious bronchitis virus, avian infectious
  laryngotracheitis virus, chicken Marek's disease virus, mycoplasma gallisepticum, salmonella, and chicken genome DNA.
- Wide Applicability: Can be paired with various fluorescent quantitative PCR instruments on the market, especially supporting Bioer Real-Time PCR instruments.
- **Good Stability:** The reagent kit adopts the form of lyophillization. After lyophillization, the active substances (such as enzymes) in the product will be protected at ambient temperature, and can be transported at room temperature, improving and extending the stability of the reagent, greatly reducing the problems of reagent performance degradation or failure caused by temperature changes.
- Simple Operation and Short Time Consumption: The reagent kit does not need to be prepared, and after re-dissolution, it only needs to be packaged in one step to add samples for testing, and the test results can be obtained within 1 hour.

#### **Application Cases**

#### Case 1

Specificity detection of avian influenza virus nucleic acid detection kit H1N1, H3N2, H5N1, H7N9, H9N2, influenza B virus, Newcastle disease virus, infectious bursa of fabricius disease virus, avian infectious bronchitis virus, avian infectious laryngotracheitis virus, chicken marek's disease virus, mycoplasma gallisepticum, salmonella, and chicken genomic DNA were detected with this kit, and the results showed that all samples were negative except H5N1, H7N9, and H9N2, This indicates that the reagent kit has no cross reaction with other viruses and has good specificity.



Figure 1.Amplification curve of the cross-validation experiment of the reagent kit

Table 1. Summary of cross-validation results for reagent kits

Sample	H5	H7	H9
H5N1	+	-	-
H7N9	-	+	-
H9N2	-	-	+
H1N1	-	-	-
H3N2	-	-	-
Influenza B Virus	-	-	-
Newcastle Disease Virus	-	-	-
Infectious Bursa of Fabricius Virus	-	-	-
Avian Infectious Bronchitis	-	-	-
Avian infectious Laryngotracheitis Virus	-	-	-
Chicken Marek's Disease Virus	-	-	-
Mycoplasma Gallisepticum	-	-	-
Salmonella	-	-	-
Chicken Genome DNA	-	-	-

Note: "+" represents a positive result, and "-" represents a negative result.

10<sup>4</sup> copies/mL of avian influenza H5N1, H7N9, and H9N2 positive samples were repeat 10 times using the avian influenza virus H5/H7/H9 subtype Lyophilized nucleic acid detection kit (fluorescence PCR method). After statistical analysis, it was found that the co-efficient of variation (CV) of Ct values for each subtype detection result was less than 1%, indicating that the precision of the kit was good and the experimental results were reliable.



NO.	H5	H7	Н9
1	29.37	29.96	28.18
2	29.4	29.76	28.07
3	29.61	29.87	28.16
4	29.11	29.72	28.19
5	29.32	29.58	28.11
6	29.52	29.71	28.05
7	28.91	29.59	28.12
8	28.75	29.42	28.06
9	29.52	29.89	28.35
10	29.15	29.59	28.07
CV	0.96%	0.56%	0.32%

Figure 2. Amplification Curve of Repeatability Verification Experiment

Table 2. Summary of Repeatability Verification Results of the Kit

## Avian Influenza Virus H5N8 Subtype Nucleic Acid Detection Kit (Fluorescence RT-PCR)

#### Introduction

Avian Influenza Virus (AIV) is a kind of influenza A virus, which is listed as a severe infectious disease of type A by the World Organization for Animal Health (Founded as OIE). According to the antigenicity of the outer membrane hemagglutinin (H) and neuraminidase (N) proteins, it can be divided into 16 H subtypes (H1-H16) and 9 N subtypes (N1-N9). Among AIV, H5N1/H7N7/H9N2 subtypes can infect humans. The high fatality rate of H5N8 subtype makes poultry products seriously unsalable, farmers suffer heavy losses, and cause great economic losses to the poultry farming industry.

#### **Bioer AIV Detection Method**

RT-PCR technology has the advantages of high specificity and sensitivity, and the real-time fluorescent RT-PCR method is recommended for laboratory pathogen nucleic acid detection. The detection of AIV is not only used for disease diagnosis, epidemiological investigation, and pathogenesis research, but also can diagnose multiple subtypes and distinguish different subtypes. Based on its own mature molecular diagnosis platform and technical reserves, Bioer has developed related products of AIV nucleic acid detection reagents according to the needs of different customer.



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Product Name	Catalogue Number	Packing Size (Tests/Kit)	Detect Target	Storage Condition
Avian Influenza Virus H5N8 Subtype Nucleic Acid Detection Kit (Fluorescence RT-PCR)	BSL54S1	24T	AIV H5&N8 subtype	-20°C
Avian Influenza Virus H5N8 Subtype Nucleic Acid Detection Kit (Fluorescence RT-PCR)	BSL54M1	48T	AIV H5&N8 subtype	-20°C

- Sample Type: Available for animal plasma, serum, swab, tissue and virus culture.
- High Sensitivity: The detection limit can reach 500copies/mL.
- High Specificity: It can distinguish different subtypes of AIV, and maintain good negative results for the detection of Avian influenza B.
- Wide Applicability: It can be used in various Real-Time PCR instruments on the market, especially supporting Bioer Real-Time PCR instruments.
- Simple Operatation and Short Time Consuming: The kit adopts premixed form, with few components, which is convenient for operation; the PCR reaction process can be completed in only 1 hour.

#### **Application Cases**

#### Case 1

Using this kit to detect the H5N8 positive sample and make standard curve, which find the correlation coefficients of the Ct values detected by each subtype are above 0.995, indicating that the kit has a good linear relationship and high PCR efficiency. The results are as follows:



#### Case 2: AIV H5N8 Subtype Nucleic Acid Detection Kit Specificity Verification

Using this kit to detect positive samples of H1N1, H3N2, H5N1, H5N8, H7N9, H9N2, B-type V strain, B-type Y strain, Newcastle disease virus, duck plague virus, chicken infectious bursal virus, chicken infectious bronchitis virus respectively. The results showed that the kit could only detect the H5 subtype in the above samples, and the rest of the samples were all negative; indicating that the kit had no crossover with the above viruses, and the specificity was good. The results are summarized as follows:



Sample	FAM	HEX
H1N1	NoCt	NoCt
H3N2	NoCt	NoCt
H5N1	17.42	NoCt
H5N8	31.87	26.58
H7N9	NoCt	NoCt
H9N2	NoCt	NoCt

	Sample	FAM	HEX
t	B-type V strain	NoCt	NoCt
t	B-type Y strain	NoCt	NoCt
t	Newcastle disease virus	NoCt	NoCt
3	Duck plague virus	NoCt	NoCt
t	Chicken infectious bursal virus	NoCt	NoCt
t	Chicken infectious bronchitis virus	NoCt	NoCt

#### II Case 3

The kit was stored at -20°C and 37°C for 7 days, and was taken out to detect positive samples of different concentrations. The results showed that the kits being stored at 37°C for 7 days, compared to the kits being stored at -20°C to detected samples of different concentrations at the same time, there was no significant difference in the detection effect, indicating that the stability of the kit was good. The detection result is as follows:



Nucleic	-20	0°C	C freeze-th		
Acid Sample	FAM	HEX	ROX	FAM	
Sample 1	20.79	19.04	20.79	19.36	
Sample 2	24.19	23.16	24.34	23.21	
Sample 3	27.83	27.02	27.84	26.63	
Sample 4	32.00	31.28	31.89	31.34	
Sample 5	34.61	33.59	33.95	33.54	



## Avian Influenza Virus H7N9 Subtype Nucleic Acid Detection Kit (Fluorescent PCR method)

#### Introduction

Avian influenza virus (AIV) is a type of influenza A virus and is classified as a class A severe infectious disease by the World Organization for Animal Health (OIE). According to the different antigenicity of its outer membrane hemagglutinin (H) and neuraminidase (N) proteins, it can be divided into 16 H subtypes (H1~H16) and 9 N subtypes (N1~N9). Among the avian influenza viruses, H5N1, H7N7, and H9N2 can be transmitted to humans. Among them, the high fatality rate of the H7N9 subtype makes poultry products seriously unsalable, and farmers have suffered heavy losses, which has caused great economic losses to the poultry farming industry.

#### **Bioer Technology Avian influenza Virus Detection Method**

RT-PCR technology has the advantages of high specificity and sensitivity. Real-time fluorescence RT-PCR is recommended for laboratory pathogen nucleic acid detection. The detection of avian influenza virus is not only used for the diagnosis of diseases, epidemiological investigations, and pathogenesis research, but also for the diagnosis of multiple subtypes and the distinction between different subtypes. Based on its own mature molecular diagnostic platform and technical reserves, Bioer Technology has developed products related to avian influenza virus nucleic acid detection reagents for the needs of different customer groups.

Product Name	Catalogue Number	Packing Size (Tests/Kit)	Detect Target	Storage Condition
Avian Influenza Virus H7N9 Subtype Nucleic Acid Detection Kit (Fluorescent PCR method)	BSL57S1	24T	Avian influenza virus H7N9 subtype	-20°C
Avian Influenza Virus H7N9 Subtype Nucleic Acid Detection Kit (Fluorescent PCR method)	BSL57M1	48T	Avian influenza virus H7N9 subtype	-20°C

#### **Reagent kit characteristics**

- Sample Types: Animal plasma, serum, swabs, tissues and virus cultures can be selected.
- Ultra-high Sensitivity: The detection limit can reach 500copies/mL.
- High Specificity: It can distinguish the subtypes of avian influenza virus, and all tests for influenza B have maintained good negative results.
- Wide Applicability: It can be used for various fluorescence quantitative PCR instruments on the market, especially for the existing fluorescence quantitative PCR instruments of Bioer Company.
- Simple Operatation and Short Time Consuming: The kit adopts the form of premix, with few components, which is convenient for operation; the PCR reaction process can be completed in just 1h

#### **Application cases (or experimental results)**

#### II Case 1

## Specific detection of avian influenza virus H7N9 subtype nucleic acid detection kit

Positive samples of H1N1, H3N2, H5N1, H7N9, H9N2, Type B V, Type B Y, Newcastle disease virus, duck fever virus, chicken infectious Farsi virus, and chicken infectious bronchitis virus were tested using a kit. The results showed that the H7N9 subtype of avian influenza can detect H7 subtype and N9 subtype samples, and the rest of the samples were negative; it shows that the kit has no cross with the above viruses and has good specificity. The results are summarized as follows:

6800	Sample	FAM	HEX	Sample	FAM	HEX
6400- 6000- 5600-	Type A H1N1	NoCt	NoCt	Type A H1N1	NoCt	NoCt
8000 9 4000 9 4000 9 4000	Type A H3N2	NoCt	NoCt	Type A H3N2	NoCt	NoCt
	Type A H5N1	NoCt	NoCt	Type A H5N1	NoCt	NoCt
2400	Avian influenza H9N2	NoCt	NoCt	Avian influenza H9N2	NoCt	NoCt
1200- 800- 400-	Avian influenza H7N9	26.14	28.18	Avian influenza H7N9	26.14	28.18
0 2 4 6 8 10 12 14 16 13 20 22 54 58 25 20 22 34 38 39 40 42 44 46 Cycles	Type B V series	NoCt	NoCt	Type B V series	NoCt	NoCt

#### E Case 2

The positive samples of avian influenza virus H7N9 were repeatedly tested with this kit 20 times. After statistics, it was found that the CT value coefficient of variation (CV) of the test results of each subtype was less than 1%, indicating that the precision of the kit is good, and the test results of the same sample are stable and reliable. The specific results are summarized as follows:

į	500-	Sample	FAM	HEX	Sample	FAM	НЕХ
1	5700	1	31.13	31.80	11	30.98	31.93
		2	31.19	31.97	12	30.83	32
	1900	3	30.87	31.85	13	30.99	32.04
scenc	600- 	4	30.96	32.02	14	30.97	31.68
Fluore	5000	5	30.62	31.66	15	30.92	31.74
:	2400-	6	20.05	21.05	16	31.01	31.68
	1500-	0	30.93	31.03	17	30.85	32.1
	200-	/	30.84	31.47	18	30.9	31.79
	600- 200-	8	31.00	31.89	19	30.92	31.91
		9	31.08	31.97	20	30.71	31.85
	Cycles	10	30.78	31.64	CV	0.44%	0.50%

The kit was placed at -20°C and 37°C for 7 days, and after being removed, it was used to detect positive samples of different concentrations. The results showed that: after the kit was placed at 37°C for 7 days, when the reagent placed at -20°C was used to detect samples of different concentrations at the same time, the detection effect was not significantly different, indicating that the stability of the kit is good. The detection effect is as follows:



Nucleic	-20	0°C	37°C acceleration for 7 days		
cid Sample	FAM	HEX	FAM	HEX	
1	23.69	25.56	23.63	25.98	
2	27.07	29.00	27.03	29.24	
3	30.63	32.51	30.66	32.81	
4	34.48	35.35	34.69	36.73	

## Avian Influenza Virus H5N1 Subtype Nucleic Acid Detection Kit (Fluorescent PCR method)

#### Introduction

Avian influenza virus (AIV) is a type of influenza A virus and is classified as a class A severe infectious disease by the World Organization for Animal Health (OIE). According to the different antigenicity of its outer membrane hemagglutinin (H) and neuraminidase (N) proteins, it can be divided into 16 H subtypes (H1~H16) and 9 N subtypes (N1~N9). Among avian influenza viruses, H5N1, H7N7, and H9N2 can be transmitted to humans, of which H5N1 is highly pathogenic and has a high fatality rate. Avian influenza has caused serious unsalable poultry products, caused heavy losses to farmers, and caused great economic losses to the poultry farming industry.

#### **Bioer Technology Avian influenza Virus Detection Method**

RT-PCR technology has the advantages of high specificity and sensitivity. Real-time fluorescence RT-PCR is recommended for laboratory pathogen nucleic acid detection. The detection of avian influenza virus is not only used for the diagnosis of diseases, epidemiological investigations, and pathogenesis research, but also for the diagnosis of multiple subtypes and the distinction between different subtypes. Based on its own mature molecular diagnostic platform and technical reserves, Bioer Technology has developed products related to avian influenza virus nucleic acid detection reagents for the needs of different customer groups.

Product Name	Catalogue Number	Packing Size (Tests/Kit)	Detect Target	Storage Condition
Avian Influenza Virus H5N1 Subtype Nucleic Acid Detection Kit (Fluorescent PCR method)	BSL58S1	24T	Avian influenza virus H5N1 subtype	-20°C
Avian Influenza Virus H5N1 Subtype Nucleic Acid Detection Kit (Fluorescent PCR method)	BSL58M1	48T	Avian influenza virus H5N1 subtype	-20°C

- Sample Types: Animal plasma, serum, swabs, tissues and virus cultures can be selected.
- Ultra-high Sensitivity: The detection limit can reach 500copies/mL.
- High Specificity: It can distinguish the subtypes of avian influenza virus, and all tests for influenza B have maintained good negative results.
- Wide Applicability: It can be used for various fluorescence quantitative PCR instruments on the market, especially for the existing fluorescence quantitative PCR instruments of Bioer Company.
- Simple Operation and Short Time Consuming: The kit adopts the form of premix, with few components, which is convenient for operation; the PCR reaction process can be completed in just 1h.

#### Application cases (or experimental results)

#### I Case 1

Use this kit to test positive samples of the H5N1 virus and draw a standard curve. It was found that the correlation coefficient of the CT values detected by each subtype reached more than 0.995, indicating that the linear relationship of the kit is good and the PCR efficiency is high. The results are as follows:



#### Case 2 Specific detection of avian influenza virus H5N1 subtype nucleic acid detection kit

Positive samples of H1N1, H3N2, H5N1, H7N9, H9N2, Type B V, Type B Y, Newcastle disease virus, duck fever virus, chicken infectious Farsi virus, chicken infectious bronchitis virus were tested using a kit. The results showed that the H5N1 subtype of avian influenza could detect H5 subtype and N1 subtype samples, and the rest of the samples were negative; It shows that the kit has no crossover with the above viruses and has good specificity. The results are summarized as follows:

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Sample	FAM	HEX
H1N1	NoCt	29.09
H3N2	NoCt	NoCt
H5N1	24.47	22.55
H7N9	NoCt	NoCt
H9 subtype vaccine	NoCt	NoCt
Type B Y series	NoCt	NoCt

Sample	FAM	HEX
Type B V series	NoCt	NoCt
Newcastle Disease Virus	NoCt	NoCt
Duck fever virus	NoCt	NoCt
Chicken infectious Farsi cystic virus	NoCt	NoCt
Chicken infectious bronchitis virus	NoCt	NoCt

#### II Case 3

The positive samples of avian influenza H5N1 were repeatedly tested 20 times using the kit. After statistics, it was found that the coefficient of variation (CV) of the CT value of the test results of each subtype was less than 1%, indicating that the precision of the kit is good, and the test results of the same sample are stable and reliable.The specific results are summarized as follows:

Sample	FAM	HEX	Sample	FAM	HEX
1	30.96	29.42	11	30.28	29.38
2	30.62	29.46	12	30.34	29.19
3	30.48	29.29	13	30.32	29.21
4	30.33	29.26	14	30.56	29.12
5	30.61	29.23	15	30.74	29.24
	00.04	20.20	16	30.46	29.11
6	30.64	29.29	17	30.83	29.36
7	30.34	29.14	18	30.76	29.37
8	30.37	29.31	19	30.84	29.43
9	30.55	29.43	20	30.9	29.21
10	30.58	29.31	CV	0.69%	0.37%

#### II Case 3

The kit was placed at -20°C and 37°C for 7 days, and after being removed, it was used to detect positive samples of different concentrations. The results showed that: after the kit was placed at 37°C for 7 days, when the reagent placed at -20°C was used to detect samples of different concentrations at the same time, the detection effect was not significantly different, indicating that the stability of the kit is good. The detection effect is as follows:



Sample	-20°C		37°C		
	FAM	HEX	FAM	HEX	
1	23.79	22.09	24.58	22.71	
2	26.79	25.31	27.61	25.74	
3	30.16	28.62	30.86	29.00	
4	33.33	31.90	33.93	32.05	

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