Avian Influenza Virus (AIV) H5-H7-H9 RT-PCR Detection

One-step real-time TaqMan Probe RT-PCR for AIV H5, H7and H9 Detection from avian samples

Catalog	Kit Size (RXN)
TBS42039	100

DESCRIPTION

Avian Influenza Virus (AIV) TaqMan Probe RT-PCR Detection Kit is one step real-time reverse transcription polymerase chain reaction (RT-PCR) assay intended for the simultaneous detection of AIV H5, H7 and H9 subtypes in avian samples in one reaction tube. It combines both reverse transcription (RT) and Taqman probe-based PCR amplification to occur in a single reaction tube. The kit is optimized for the two reactions in a real-time "single step". This One-step qRT-PCR assay offers the end-users an efficient, easy to use, and reliable alternative to conventional "two-step" sequential qRT-PCR for detecting targets.

AIV TaqMan Probe RT-PCR Detection Kit contains all components for RT-PCR reaction including RT-PCR enzymes, primer-probes, internal control, positive control, negative control, and buffer. The AIV target genes H5, H7, and H9 are labeled with fluorescence Fam, and internal control is labeled with Hex.

APPLICATIONS

This kit is used for the detection of AIV H5, H7, and H9 subtypes in avian samples.

KEY FEATURES

- One-step complete qRT-PCR in a single tube.
- Reduce contamination in the operating process.
- Accurate and simultaneous detection of AIV H5, H7, and H9 subtypes.
- 4x RT-PCR mix makes it easier to adjust the sample size in a one-tube reaction.

KIT CONTENTS

Component	100RXN
4X RT-PCR Master Mix	0.5 mL
10X AIV Primer-probe Mix	0.2 mL
AIV Positive Control	100 µL
AIV Negative Control	100 µL
4x RT-PCR Buffer	0.5 mL
DEPC Water	1.0 mL

STORAGE CONDITIONS

Store all components at -20°C in a non-frost-free freezer.
Shelf life is 12 months after recipient.
The kit is shipped on ice.

PROTOCOL

Precautions: RT-PCR should be assembled in a nuclease-free environment. RNA sample preparation, reaction mixture assembly, PCR and subsequent reaction analysis should be performed in separate areas. The use of "clean", automatic pipettors designated for PCR and aerosol resistant barrier tips are recommended.

Note: Nuclease Removal (TBS6013) is useful for removing RNase contamination in the working area and tools.

1. RNA isolation is performed with a suitable approach. We recommend our Hybrid-R RNA purification kit (catalog: 305-101) for RNA extraction from avian samples.

2. Prepare the following reaction mixture on ice:

Components	Reaction Vol.:20 µl	Concentration
Total RNA	Variable	10 pg - 1 µg/rxn
RT-PCR Mix (4x)	5 µl	1X
Primer-probe Mix (10x)	2 µl	1x
RT-PCR Buffer (4x)	5 µl	1x
DEPC water	Adjust to the final volume to 20	

Positive or Negative Control: 2 µl/well.
The AIV H5, H7, and H9 are labeled with Fam, and internal control is labeled with Hex

Note:

1. Gene specific primers and probe must be used for RT-qPCR amplification.
2. Please ensure no salt crystals are present in the RT-qPCR Mix before use. If salt crystals are observed, mix until crystals are completely dissolved and absent.
3. For Positive and negative control, use 2 µl of Positive or negative control to replace the RNA sample in positive or negative control wells.

3. Gently mix and ensure all the components are at the bottom of the amplification tube. Centrifuge briefly if needed.
4. Program the thermal cycler so that cDNA synthesis is followed immediately by qPCR amplification.

Step	Temperature	Duration	Cycle(s)
1	50°C	20 mins	1
2	95°C	1 min	1
3	95°C	15 secs	40
	60°C	60 secs	

Recommendations for Optimal Results

- Aliquot reagents to avoid contamination and repeated freeze-thaw cycles.
- Start reaction as soon as the reaction mixture is prepared and always keep the reaction mixture chilled in an ice box prior to qRT-PCR reaction.

DATA ANALYSIS

After completion of the run, save and analyze the data following the instrument instructions. Analyses should be performed separately for each fluorescence channel using a manual threshold setting. Thresholds should be adjusted to fall within exponential phase of the fluorescence curves and

above any background signal. The procedure chosen for setting the threshold should be used consistently.

The results can be interpreted as below:

Positive: AIV H5, H7, and H9 target Ct value is in 12-37, and internal control (Hex), Positive control, and Negative control are normal.

Negative: AIV gene Ct value is ≥38, and internal control (Hex), Positive control, and Negative control are normal.

RELATED PRODUCTS

TBS6025: Microbial DNA Magnetic Extraction
TBS42018: Trichothecene-producing Fusarium Species TaqProbe qPCR Detection
TBS42019: Fusarium Species qPCR Detection
TBS42020: Universal Aspergillus qPCR
TBS42021: Aspergillus Flavus qPCR
TBS42022: Aspergillus Fumigatus qPCR
TBS42023: Aspergillus Niger qPCR
TBS42024: Aspergillus Terreus qPCR
TBS42025: 4-In-1 Aspergillus qPCR
TBS42026: O157H7 E. Coli qPCR
TBS42027: STEC qPCR
TBS42028: Salmonella qPCR
TBS42029: STEC and Salmonella Multiple qPCR
TBS42030: Mycoplasma Detection qPCR
TBS42031: Listeria Monocytogenes qPCR
TBS42032: Listeria Genus qPCR
TBS42033: Bacillus Cereus qPCR
TBS42043: Bacillus Species qPCR

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