Instruction manual
ADI531-H5H7_NO_(EN)_V01
11/2022

ADIAVET™ AIV H5-H7 REAL TIME

Reference: ADI531-100

Test for the detection of Avian Influenza Virus subtypes H5 and H7 by real time enzymatic amplification
PCR Test – 100 reactions

For veterinary *in vitro* use only

Sample	Individual analysis	Pool of sample possible*, up to:
Swab (tracheal, cloacal…)	✓	5
Tissue (lung)	✓	5
Environmental samples (drag swab)	✓	✗
Feather	✓	5
FTA cards	✓	✗
Faeces	✓	✗
Culture / allantoic fluid	✓	✗

* Depending on the epidemiological case and on the quality of samples. **Depending on the country AI detection is subject to specific directives.**

Kit composition

Content		ADI531-100 Kit 100 reactions
A5	Amplification solution	2 x 1000 µL tube with green cap (Ready to use)
H5-H7 CTL+	AIV H5 and AIV H7 positive control	1 tube with purple cap (To reconstitute)
EPC-Ext	Exogenous extraction control	1 x 1250 µL tube with yellow cap (Ready to use)
NF-Water	Nuclease-Free Water	1 x 1000 µL tube with white cap (Ready to use)

Revision history

Date	Version	Modifications
01/2020	NE531-03	
11/2022	ADI531-H5H7_NO_(EN)_V01	New instruction template Addition of EPC-Ext for environmental and wild bird samples

Note: minor typographical, grammar and formatting changes are not included in the revision history.

A. Introduction

Avian influenza (AI) viruses belong to the Influenza virus A genus of the Orthomyxoviridae. family. They are negative single stranded RNA viruses divided into subtypes based on two surface proteins: hemagglutinin and neuraminidase. Today, there are 16 subtypes of hemagglutinin (H1-H16) and 9 subtypes of neuraminidase (N1-N9) described.

AIV can cause severe diseases in domestic poultry, including chickens and turkeys but can also infect pheasants, quails, ducks, geese…

Strains of avian influenza virus are classed as low or highly pathogenic. An influenza virus is classed as highly pathogenic if one of the following criteria is verified:

- Determination of pathogenicity index by intravenous (IVPI) greater than 1.2
- Presence of an amino acid sequence of the cleavage site of haemagglutinin similar to a sequence already observed for highly pathogenic IA isolates (presence of several basic amino acids).

All H5 and H7 subtypes (low and highly pathogenic) must be declared to the WOAH.

Real-time PCR could be a method to obtain result within one day, with a high specificity and sensitivity.

B. Test principle

ADIAVET™ AIV H5-H7 REAL TIME test is based on the reverse transcription (RT) of RNA into complementary DNA. This reaction is followed by gene amplification of subtypes H5 and H7 of Avian Influenza Virus (AIV) specific DNA fragments. This test is intended to detect simultaneously, in one well:

- Subtype H5 Avian influenza virus (FAM labelled probe).
- Subtype H7 Avian influenza virus (Cy5 labelled probe).
- GAPDH internal control of extraction and amplification specific from an endogenous nucleic acid (HEX labelled probe or its equivalent).

C. Storage conditions

After reception, the kit should be stored at <-15 °C until the expiration date.

It is recommended to make aliquots of A5 solution if it should be defrosted more than 3 times.

Do not thaw more than 3 times.

Store away from sunlight.

Do not mix reagents from two different batches.

D. Material required but not provided

- Real-time Thermal cycler and device.
- Instrument for homogenous mixing of tubes.
- Pipettes of 1 - 10 µL, 20 - 200 µL and 200 - 1000 µL.
- Nuclease-free filtered pipette tips.
- Nuclease-free microtubes of 1,5 mL and 2 mL.
- Powdered-free latex or nitrile gloves.
- Nuclease-free water.
- Kit for nucleic acids extraction.

Additional kits for method adoption and PCR

- **Extraction Positive Control AIV&H5-H7 (Ref.: ADC28EPC).** Supplier reference material for method adoption that can also be used as a sentinel (Calibrated between 1 and 100xLOD_{Method}).
- **LD_{PCR} Positive Control – AIV H5-H7 (Ref.: ADC53LD)** Confirmation of performances – LOD_{PCR} of kit.

E. Warnings and precautions

- For veterinary *in vitro* use only.
- For animal use only.
- For professional use only.
- All instructions must be read before performing the test and strictly respected.
- Do not use reagents after the expiration date.
- Do not use reagents if the packaging is damaged.
- Do not open PCR wells or tubes after amplification.
- Do not mix reagents from different batches.
- Used material must be disposed of in compliance with the legislation in force regarding environmental protection and biological waste management.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).

F. Nucleic acids extraction

1. Extraction kits

Nucleic acids must be extracted from the samples before using the kit. The RNA/DNA purification kits listed below are recommended by Bio-X Diagnostics:

Product name	Extraction system	Number of tests and reference
ADIAMAG	Magnetic beads	200 tests: ref. NADI003 800 tests: ref. NADI003-XL

For the extraction, consult the user manual version, available on the website, indicated on the certificate of analysis included in the PCR kit of interest.

Extraction protocols are described in validation data. Other purification kits can be used if they have been validated by the user.

After extraction, nucleic acid extracts can be kept on ice or at +2/8 °C for a few hours or until use. For long term storage, they must be kept at a temperature below -15 °C or -65 °C.

2. Controls

Using controls allow to verify the reliability of the results. Controls can be included by series of analysis according to the recommendations defined by the standards in force (Cf. AFNOR U47-600…).

Controls	Validation of	How to proceed
No Template Control (NTC)	Absence of amplification contamination	5 µL NF-Water in a well per run
H5-H7 CTL+	AIV and IPC target amplification	5 µL CTL+
Negative extraction control	Absence of contamination for the extraction and amplification	1 extraction (water or lysis buffer) per run
Positive extraction control	Extraction and amplification	1 extraction (Positive sample between 1 et 100X LOD_{METHOD}) per run

G. Procedure

1. Use of EPC-Ext

EPC-Ext must be added to environmental samples or wild / exotic bird samples (other than chicken, turkeys, ducks, or geese).

- Aliquot and store the solution at a temperature below -15 °C according to the size of extraction series. Do not thaw more than 3 times.

- Add **5 µL** of EPC-Ext in the first nucleic acids extraction lysis buffer.

2. Use of CTL+

- Add **200 µL** of **« NF-Water »** per tube.
- Homogenize the tubes using a mixer, such as vortex, > 20 seconds and until dissolution of the blue pellet.
- After reconstitution, aliquot and store the solution at a temperature below -15 °C until the kit expiration date. Do not thaw more than 3 times.
- For each assay, use **5 µL** of CTL+ in one of the dedicated wells (see § « Amplification », Step 2).

3. Amplification

Warning:

- Before starting, rehydrate or thaw reagents at room temperature in the dark.
- Homogenize all reagents and samples before use.
- Store reagents at a temperature below -15 °C after use.

Step 1: Dispense **20 µL** of amplification solution (A5) per well.
Step 2: Dispense **5 µL** of nucleic acids extracts and **5 µL** of controls in each dedicated well.
Use NF-Water for the No Template Control (NTC).
Step 3: Cover the wells with an appropriate optical film or caps.
Step 4: Start the PCR analysis.

The following program is defined for ABI Prism thermocyclers (like 7500, QuantStudio5, Step-one…) from Applied Biosystems, for Mx3000, Mx3005P and AriaMx from Agilent, for LightCycler from Roche Diagnostics, for Rotor-Gene Q from Qiagen, for CFX96 and Chromo 4 from Biorad and for MIC from BioMolecular System.

RNA 54 °C program	
10 min. 45 °C	
10 min. 95 °C	
15 sec. 95 °C*	40 cycles
60 sec. **54 °C****	

*30 sec. 95°C for MX3000 and MX3005P
** Reading and parameters for fluorescence acquisition:

Fluorochrome	Absorbance (nm)	Emission (nm)
FAM	494	520
HEX or equivalent	530	549
Cy5	646	662
ROX	575	602

Note: The Quencher is non-fluorescent. The A5 solution contains a passive reference read in the same spectra as ROX for ABI machines.

For other thermal cycler instruments**,** please contact your sales representative or the customer relations department.

H. Interpretation of results

Display all curves and position the threshold line for each fluorochrome.

1. Test validation

Amplification is valid if the following results are obtained.
Expected Ct (Threshold Cycle*)* values for the CTL+ are indicated on the certificate of analysis of the kit.

Controls	Amplification			Validation of
	FAM	Cy5	HEX or equivalent	
No Template Control (NTC)	No	No	No	Absence of amplification contamination
H5-H7 CTL+	Yes	Yes	Yes	Amplification of H5, H7 and IPC target
Extraction negative control	No	No	Yes/No	Absence of extraction contamination
Extraction positive control	Yes	Yes	Yes/No	Extraction and amplification steps

2. Results interpretation

Nucleic acids extraction and amplification are **valid** for each sample if at least one typical amplification curve is observed in FAM, Cy5 and/or HEX or equivalent.

Amplification			Interpretation	
FAM	Cy5	HEX or equivalent	H5	H7
No	No	Yes	Undetected	Undetected
Yes	Yes	Yes/No	Detected	Detected
Yes	No	Yes/No	Detected	Undetected
No	Yes	Yes/No	Undetected	Detected
No	No	No	Undetermined	Undetermined

« Undetermined »: no characteristic amplification curve.
Possible causes:
Defective PCR due to inhibitors, set up error, absence of samples, degraded samples and/or issue with nucleic acids extraction (loss or destruction of nucleic acids).
Recommendations:
Set up a new PCR assay using pure nucleic acids extracts and 10x dilutions in Nuclease-free water.
If the assay is inconclusive, perform a new nucleic acids extraction.

Symbols

Symbols	Signification
REF	Catalog number
	Manufacturer
	Temperature limitation
	Use by
LOT	Batch code
	Consult Instructions for Use
	Contain sufficient for "n" tests
VET	For veterinary *in vitro* use only – For animal use only
	Keep away from sunlight

Notes

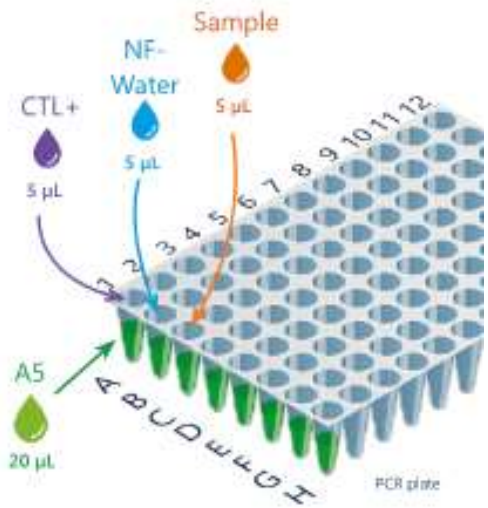
1 Extract nucleic acids with

Adia X Mag →

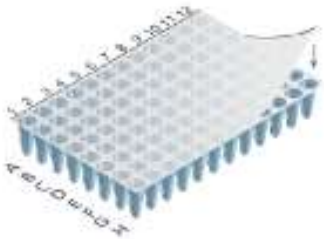
Scan me to discover Adiamag™

2 Distribute 20 µL of A5 amplification solution

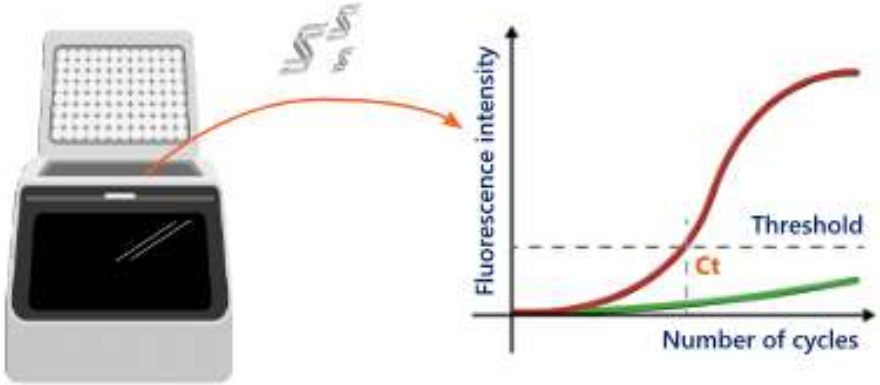
3 Distribute **5 µL** of nucleic acids, CTL+ and NF-Water



4 Seal the wells



5 Start PCR analysis



*The notes do not replace the instructions for use of which they are a summary.

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