Instruction manual
ADL65Y1_IBV_NO_(EN)_V02
01/2025

IBV

Reference: ADL65Y1-100

Test for the detection of *Infectious Bronchitis Virus* 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 (Cloacal, tracheal, oropharyngeal)	✓	6
Environmental sample	✓	✗
FTA card	✓	✗

*Depending on the epidemiological case and on the quality of samples.

Kit composition

Content		ADL65Y1-100 Kit
		100 reactions
A6	Amplification solution	1 lyophilized vial with blank caps (To reconstitute)
Rehydration buffer	Rehydration solution	1 x 6 mL vial (Ready to use)
IBV CTL+	IBV positive control	1 tube with purple cap (To reconstitute)
EPC-Ext	Exogeneous or amplification extraction control	1 lyophilized vial with yellow cap (To reconstitute)
NF-Water	Nuclease-Free Water	2 x 1000 µL tubes with white cap (Ready to use)

Revision history

Date	Version	Modifications
01/2023	V01	First version
01/2025	V02	Addition of FTA matrix

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

A. Introduction

Infectious Bronchitis Virus (IBV) is a highly contagious viral disease causing respiratory, reproductive or kidney problems in chickens. It is mainly characterized by respiratory signs in growing chickens. In hens, a decrease in egg production and quality can be observed. Several strains of the virus are nephropathogenic and can cause interstitial nephritis and death.

The disease is transmitted by air, by direct contact between chickens or indirectly by mechanical spread.

IBV is a coronavirus belonging to the genus gammacoronavirus and species Avian coronavirus. The IBV genome consists of a positive single-stranded RNA of approximately 27.5 kb.

Both vaccine and field strains of IBV can persist in the cecal tonsils of the intestinal tract and be excreted in the faeces for weeks or longer in clinically normal chickens.

B. Test principle

ADIALYO™ IBV test is based on the reverse transcription (RT) of RNA into complementary DNA. Then, cDNA is amplified with a DNA polymerase using specific primers of Infectious Bronchitis Virus. Both enzymatic reactions occur in the same tube (One-step RT-PCR). This test is intended to detect simultaneously, in one well:

- Infectious Bronchitis Virus (IBV) (FAM labelled probe).
- Exogenous internal control (HEX labelled probe or its equivalent).
  - Either extraction and amplification control if the EPC-Ext is added to each specimen during nucleic acids extraction steps.
  - Or amplification control if the EPC-Ext is added to A6 solution.

C. Storage conditions

- Store the kit at a temperature below +2/8 °C after reception.
- Store away from sunlight and keep dry.
- After reconstitution, prepare aliquots and store them at a temperature below -15 °C until the expiration date.
- Do not thaw more than 3 times.

D. Material required but not provided

- Thermal cycler with consumables for real-time PCR.
- Class II Microbiological Safety Cabinet.
- Centrifuge for microtubes, tubes of 15 mL.
- Instrument for homogenous mixing of tube.
- 1 - 10 µL pipette, 20 - 200 µL pipette and 200 - 1000 µL pipette.
- Nuclease-free filter tips.
- Nuclease-free microtubes: 1,5 mL and 2 mL.
- Sterile tube of 5, 10 or 15 mL.
- Latex or nitrile powder-free gloves.
- 96-100 % ethanol solution.
- Nuclease-free water.
- PBS 1X buffer (pH = 7.4).
- Kit for nucleic acids extraction.

Additional kits for method adoption and PCR

- **LOD_{PCR} Positive Control – IBV (Ref.: ADC65YLD)**

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 PCR 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
ADIAPURE™ Lysis Flex	Direct lysis for swab samples	500 mL: ref. ADPLF1-500

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 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.

Control	Validation of	Usage
No Template Control (NTC)	Absence of amplification contamination	5 µL NF-Water in a well per run
IBV CTL+	IBV target amplification	5 µL CTL+ in a well per run
Negative extraction control	Absence of contamination for the extraction and amplification	1 extraction (water or lysis buffer + EPC-Ext) per run
Positive extraction control	Extraction and amplification	1 extraction (Positive sample between 1 et 100x LOD_{METHOD}) per run

G. Procedure

1. Amplification solution A6 preparation

- Add **1000 µL** of **« Rehydration buffer »** per A6 tube.
- Homogenize tube contents using a mixer, such as vortex, at least 20 seconds.
- After reconstitution, aliquot the solution and store at a temperature below -15 °C until the expiration date. Do not thaw more than 3 times.
- To use the A6, please refer to §« Amplification », Step 1.

2. Preparation of controls

a. Use of EPC-Ext

EPC-Ext must be added to each sample and extraction controls.

- Add **1000 µL** of **« NF-Water »** per tube.
- Homogenize the tube contents using a shaker such as a vortex, > 20 seconds.
- 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 use, 2 solutions are possible:
  - add **5 µL** of EPC-Ext in the first lysis buffer during the extraction of nucleic acids in magnetic beads or silica columns.
  - Or add **0.5 µL** of EPC-Ext to each PCR well (if using ADIAPURE™ Lysis Flex direct lysis extraction). Refer to §"Amplification", Step 1.

b. 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:

If use of EPC-Ext at extraction step:

Dispense **10 µL** of amplification solution (A6) in each well.

If no use of EPC-Ext at the extraction step:

Place (n+1) x **10 µL** of amplification solution (A6) in a microtube and add (n+1) x **0.5 µL** of EPC-Ext. Dispense **10 µL** of the mixture into each well.

Step 2: Dispense **5 µL** of nucleic acids extracts and **5 µL** of controls in each dedicated well.

Use **5 µl** of NF-Water for the No Template Control (NTC).

Step 3: Cover the wells with an appropriate optical film or caps.

Step 4: Set up the amplification program.

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.

DNA/RNA Program	
10 min. 45 °C	
2 min. 95 °C	
5 sec. 95 °C	40 cycles
30 sec. 60 °C*	

*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 A6 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. Reading and interpretation

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	HEX or equivalent	
No Template Control (NTC)	No	Yes/No *	Absence of amplification contamination
IBV CTL+	Yes	Yes/No *	Target amplification
Extraction negative control	No	Yes/No *	Absence of extraction contamination
Extraction positive control	Yes	Yes	Extraction and amplification steps

*According to the addition or not of EPC during the amplification step.

2. Results interpretation

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

Amplification		Interpretation
FAM	HEX or equivalent	IBV
No	Yes	Undetected
Yes	Yes	Detected
Yes	No	Detected
No	No	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

Symbol	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
	Keep dry

Notes

1 Extract nucleic acids with

Adia X Pure → Scan me to discover Adiapure™

or

Adia X Mag → Scan me to discover Adiamag™

2 Add **1000 µL** of Rehydration buffer to the **A6** amplification solution

If using the EPC at the extraction step:

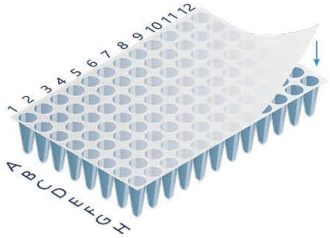
3 Distribute **10 µL** of **A6** amplification solution

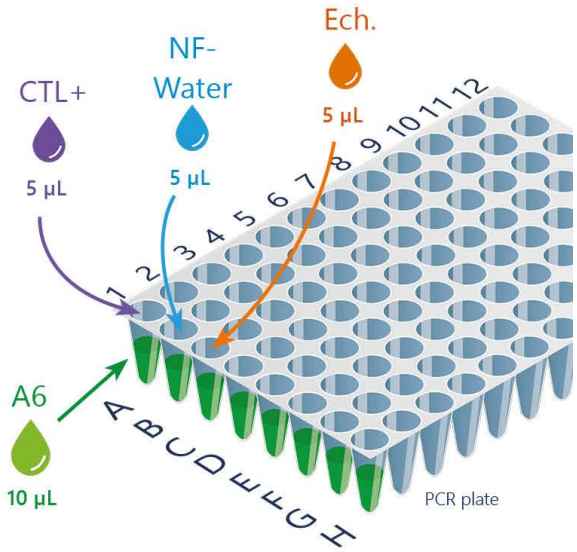
If not using the EPC at the extraction step:

3 Prepare a premix of **10 µL** of **A6** amplification solution + **0,5 µL** of EPC
Dispense **10 µL** of the premix

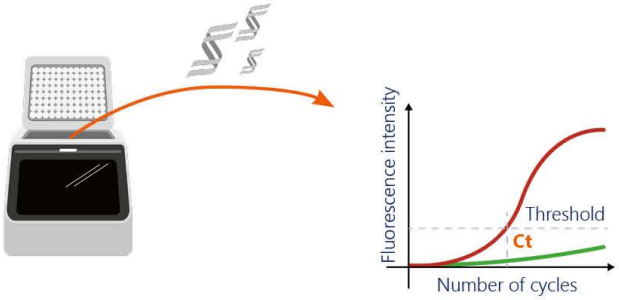
4 Distribute **5 µL of nucleic acids**, **CTL+** and **NF-Water**

5 Seal the wells





6 Start PCR analysis



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

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