Instruction manual
**ADI641-IPNV_NO_(EN)_V01**
**03/2024**

# IPNV REAL TIME

Reference: ADI641-100

Test for the detection of *infectious Pancreatic Necrosis Virus* by real time enzymatic amplification
PCR Test – 100R

For veterinary *in vitro* use only

| Sample | Individual analysis | Pool of sample possible*, up to: |
|---|---|---|
| Organ pool: spleen, kidney, heart or brain | ✓ | 10 fishes |
| Genital products (sperm, egg, coelomic liquid) | ✓ | 10 fishes |
| Culture supernatant | ✓ | ✗ |

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

## Kit composition

| Content | | ADI641-100 Kit<br>100 reactions |
|---|---|---|
| A5 | Amplification solution | 2 x 500 µL tube with green cap<br>(Ready to use) |
| IPNV CTL+ | IPNV positive control | 1 tube with purple cap<br>(To reconstitute) |
| EPC-Ext | Exogenous extraction control | 2 tubes with yellow cap<br>(Ready to use) |
| NF-Water | Nuclease-Free Water | 1 x 1000 µL tube with white cap<br>(Ready to use) |

## Revision history

| Date | Version | Modifications |
|---|---|---|
| 01/2021 | NE641-01 | First version |
| 03/2024 | ADI641-IPNV_NO_(EN)_V01 | New instruction template<br>Addition of genital products (sperm, egg, coelomic liquid) |

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

## A. Introduction

Infectious pancreatic necrosis (IPN) is a highly infectious viral disease affecting fish farms. It appears mainly in young salmonids as well as among pike fry, but almost all freshwater and saltwater fish are susceptible to disease, as are molluscs. Infectious pancreatic necrosis is present in parts of Europe, America, and Asia.

Sensitivity to NPI varies according to age since young fish are more likely to catch the disease, compared to older ones. Asymptomatic carrier parents as well as contaminated eggs are the main reservoirs of IPN. Fish can carry and transmit the infectious agent for several generations and years without showing any symptom.

IPN is caused by the infectious pancreatic necrosis virus (IPNV) which belongs to the Birnaviridae family. Aquabirnaviruses are divided into 2 serogroups based on cross-neutralization tests. In Europe, genogroups 2 and 5 are the most represented.

The standard diagnostic method has long been viral culture followed by immunological, sero-neutralization or RT-PCR identification. This method requires 2 weeks to certify virus detection failure in the culture. The use of RT-qPCR allows faster and more precise identification of the breeding status compared to viral cultivation.

## B. Test principle

ADIAVET™ IPNV REAL TIME test is based on the reverse transcription (RT) of RNA into complementary DNA. This reaction is followed by gene amplification of Infectious Pancreatic Necrosis virus specific DNA fragments. This test is intended to detect simultaneously, in one well:

- Infectious pancreatic necrosis virus (FAM labelled probe)
- Exogenous internal control (HEX labelled probe or its equivalent)

## C. Storage conditions

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

It is recommended to make fractions 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 of 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 IPNV (Ref.: ADC64EPC).**

Supplier reference material for method adoption that can also be used as a sentinel (Calibrated between 1 and 10x$LOD_{Method}$).

- **$LD_{PCR}$ Positive Control – IPNV (Ref.: ADC64LD)**

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<br>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 |
| IPNV CTL+ | IPNV 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) 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 each sample and extraction controls.

- 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. Denaturation of nucleic acids extracts

- For each sample and control(s), transfer minimum 10 µL of nucleic acids extracts in a tube or 96-plate and store the rest at a temperature below -15 °C or -65 °C.
- Incubate 3 minutes at +95 °C in a thermal cycler or heating block.
- Immediately transfer the tubes or 96-plate on melting ice or refrigerated block until use (to prevent RNA renaturation).
- To use, please refer to §« Amplification », Step 2.

### 4. 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 **10 µL** of amplification solution (A5) per well.
**Step 2:** Dispense **5 µL** of denatured 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 standard program | |
|---|---|
| 10 min. 45 °C | |
| 10 min. 95 °C | |
| 15 sec. 95 °C* | 45 cycles |
| 60 sec. 60 °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 | 538 | 554 |
| 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 | HEX or equivalent | |
| No Template Control (NTC) | No | No | Absence of amplification contamination |
| IPNV CTL+ | Yes | Yes/No | Target amplification |
| Extraction negative control | No | Yes | Absence of extraction contamination |
| Extraction positive control | Yes | Yes | 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, and/or HEX or equivalent.

| Amplification | | Interpretation |
|---|---|---|
| FAM | HEX or equivalent | IPNV |
| 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

| 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

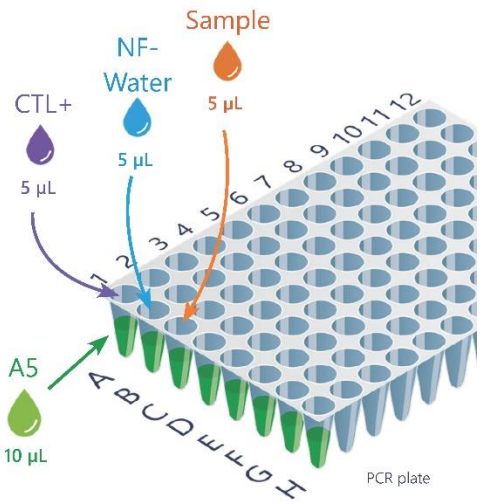
1 Extract nucleic acids with

AdiaX Mag →

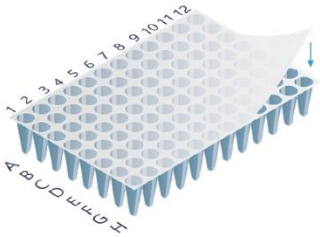
Scan me to discover Adiamag™

2 Distribute **10 µL** of **A5** amplification solution

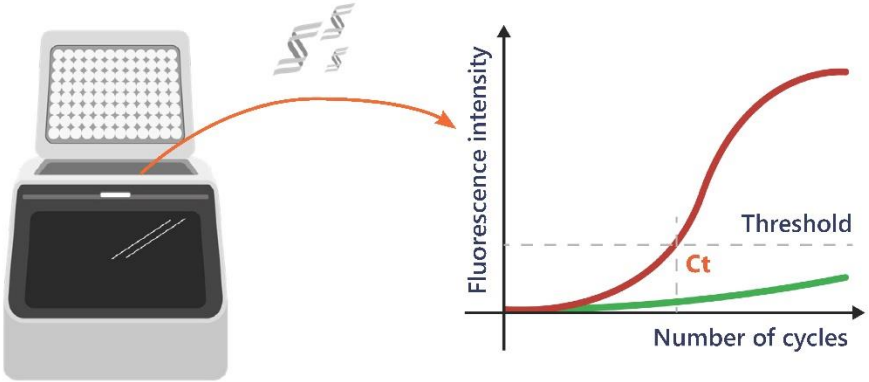
3 Distribute **5 µL** of nucleic acids, CTL+ and NF-Water




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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