
Adia X Vet

Instruction manual
**ADI381-BTV8_NO_(EN)_V02**
**11/2024**

# BTV TYPE 8 REAL TIME

Reference: ADI381-50 & ADI381-100

Test for the detection of Bluetongue Virus (BTV) of serotype 8 by real time enzymatic amplification

PCR Test – 50 & 100 reactions

For veterinary *in vitro* use only

| Sample | Individual analysis | Pool of sample possible*, up to: |
|---|---|---|
| Blood | ✓ | ✓ |
| Organs (spleen and abortive tissue) | ✓ | ✓ |

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

## Kit composition

| Content | | ADI381-50 Kit | ADI381-100 Kit |
|---|---|---|---|
| | | 50 reactions | 100 reactions |
| A5 | Amplification solution | 1 x 1000 µL tube with green cap (Ready to use) | 2 x 1000 µL tubes with green cap (Ready to use) |
| BTV T8 CTL+ | Bluetongue Virus serotype 8 positive control | 1 tube with purple cap (To reconstitute) | 1 tube with purple cap (To reconstitute) |
| NF-Water | Nuclease-Free Water | 1 x 1000 µL tube with white cap (Ready to use) | 1 x 1000 µL tube1 with white cap (Ready to use) |

## Revision history

| Date | Version | Modifications |
|---|---|---|
| 01/2020 | NE381-10 | Addition of a "NF-Water" tube in the kit |
| 06/2023 | V01 | New presentation<br>Addition of 100 reactions reference |
| 11/2024 | V02 | Addition of Organs (spleen and abortive tissue) matrix. |

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

## A. Introduction

The bluetongue virus is a non-contagious viral arthropod-borne infectious disease due to an Orbivirus (family Reoviridae, virus ARN), mainly transmitted by hematophageous midges from Culicoides genus.

The clinical expression is widely dependent on the environmental parameters (nutritional state, parasitism and bacterial infections concomitant) and on the individual sensitivity. 27 distinct serotypes exist inducing partial or no cross protections between them. Transmission by pregnant ewes has also been described. Transmission by contaminated blood injection is possible when needles and syringes are re-used.

Samples for virus detection are bloods of animals with anticoagulants (EDTA). Virus is detected by isolation on embryonated eggs, *in vitro* cell culture, immunofluorescence on cell culture or by PCR.

## B. Test principle

ADIAVET™ BTV TYPE 8 REAL TIME test is based on the reverse transcription (RT) of RNA into complementary DNA. This reaction is followed by gene amplification of Bluetongue Virus serotype 8 specific DNA fragments. This test is intended to detect simultaneously, in one well:

- Bluetongue Virus serotype 8 (FAM labelled probe)
- GAPDH: internal control of extraction and amplification specific from an endogenous nucleic acid (HEX labelled probe or its equivalent).

This test is specific to the serotype 8. No cross-reaction has been observed with other types of BTV. No cross-reaction has been observed with EHDV strains.

## 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 BTV (Ref.: ADC35EPC).** Supplier reference material used as a sentinel (Calibrated between 1 and 100x$LOD_{Method}$).
- **$LD_{PCR}$ Positive Control – BTV type 8 (Ref.: ADC38LD)** 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 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 |
| BTV T8 CTL+ | BTV 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 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 denaturated CTL+ (see § « Denaturation of nucleic acid ») in one of the dedicated wells (see § « Amplification », Step 2).

### 2. Denaturation of nucleic acids

- 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.

### 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 denatured nucleic acids extracts and **5 µL** of denatured 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 programs are 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 | | RNA FAST program | |
|---|---|---|---|
| 10 min. 45 °C | | 10 min. 45 °C | |
| 10 min. 95 °C | | 10 min. 95 °C | |
| 15 sec. 95 °C* | 40 cycles | 5 sec. 95 °C | 40 cycles |
| 60 sec. 60 °C** | | 30 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 | 530 | 549 |
| 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 |
| BTV T8 CTL+ | Yes | No | Target amplification |
| Extraction negative control | No | No | Absence of extraction contamination |
| Extraction positive control | Yes | No/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 | BTV TYPE 8 |
| No | Yes | Undetected |
| Yes, Ct < 34 | Yes | Detected |
| Yes, 34 <Ct < 40 | Yes | Detected in weakly quantities |
| No | No | Undetermined |

**« Detected in weakly quantities »:** The infection is recent or old.

**« Undetermined »:** The results can't be interpreted.
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 5x dilutions in Nuclease-free water.
If the assay is inconclusive, perform a new nucleic acids extraction by diluting the blood with a 1/2 rate in PBS. And if the result is still undetermined, the sample will be considered as non-usable (inhibitors of PCR, lysed sample…). In this case, please contact the reference laboratory or authority of your country and ask for a new sample.

**Note: This RT-PCR test does not exclude the presence of other BTV serotypes than serotype 8 in the analysed sample.**

## 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

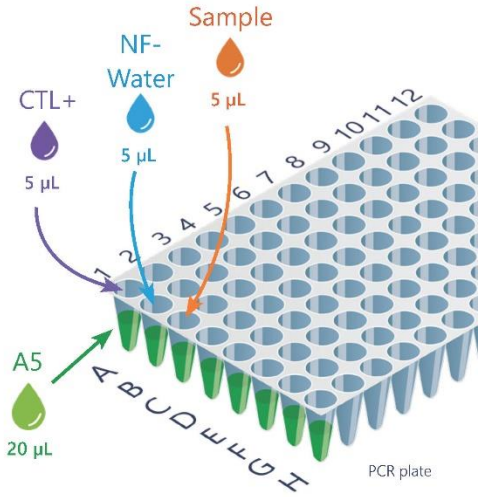
Adia X Mag

Scan me to discover Adiamag™

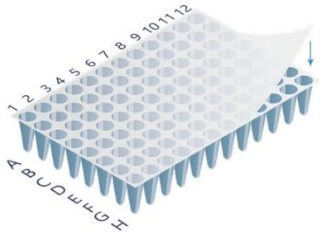
2 Distribute **20 µL** of **A5** amplification solution

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

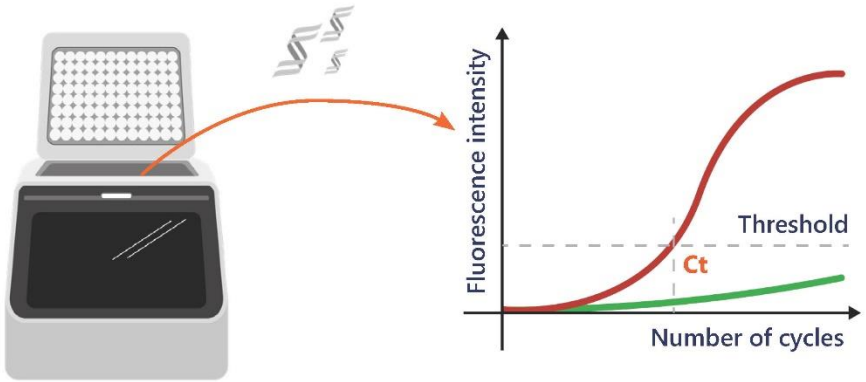
**Previously denatured 3 min. 95°C*




4 Seal the wells



5 Start PCR analysis




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




Contact us
support.pcr@biox.com
www.biox.com +32 (0) 84 32 23 77
Bio-X Diagnostics
38, rue de la Calestienne
5580 Rochefort (Belgium)