Instruction manual
ADI451-BESN_NO_(EN)_V01
09/2023

BESNOITIA REAL TIME

Reference: ADI451-100

Test for the detection of *Besnoitia besnoiti* by real time enzymatic amplification
PCR Test – 100 reactions

For veterinary *in vitro* use only

Sample	Individual analysis	Pool of sample possible*, up to:
Blood	✓	✗
Skin	✓	✗

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

Kit composition

Content		ADI451-100 Kit 100 reactions
A5	Amplification solution	2 x 1000 µL tubes with green cap (Ready to use)
Bb CTL+	Besnoitia besnoiti positive control	1 tube with purple cap (To reconstitute)
NF-Water	Nuclease-Free Water	1 x 1000 µL tube with white cap (Ready to use)

Revision history

Date	Version	Modifications
04/2021	NE451-07	Modification of the protocol of skin biopsy. Addition of extraction ADIAMAG, 800 tests, Ref. NADI003-XL.
09/2023	V01	New Layout.

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

A. Introduction

Besnoitia besnoiti, the causative agent of bovine besnoitiosis, is an obligate intracellular protozoan. The disease affects mainly young cattle. Besnoitiosis is epizootic in the south of France, but is now widely distributed in Africa, Asia and in Southwestern Europe. The most likely pathway of transmission would be transcutaneous, by stinging insects (tabanids, stomox).

Serologic tests are available for the detection of the specific antibodies of Besnoitia besnoiti present in the chronic stage. To avoid transfers of contaminated animals and to control the spread of bovine besnoitiosis, diagnostic tools allowing detection of the pathogen at the early stages of the disease are essential.

PCR tests allow detection of tachyzoites of *Besnoitia besnoiti* during febrile stage in the monocytes of blood and in the skin during oedema and chronic stages.

B. Test principle

ADIAVET™ BESNOITIA REAL TIME test is based on gene amplification of *Besnoitia besnoiti* specific DNA fragments. This test is intended to detect simultaneously, in one well:

- *Besnoitia besnoiti* (FAM labelled probe).
- GAPDH internal control of extraction and amplification specific from an endogenous nucleic acid (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

- **LD_{PCR} Positive Control – BESNOITIA (Ref.: ADC45LD)** 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
Bb CTL+	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 100 x 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 CTL+ in one of the dedicated wells (see § « Amplification », Step 2).

2. 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.

DNA standard program	
2 min. 50 °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
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
Bb CTL+	Yes	Yes	Target amplification
Extraction negative control	No	No	Absence of extraction contamination
Extraction positive control	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 and/or HEX or equivalent.

Amplification		Interpretation
FAM	HEX or equivalent	*Besnoitia besnoiti*
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
	Catalog number
	Manufacturer
	Temperature limitation
	Use by
	Batch code
	Consult Instructions for Use
	Contain sufficient for "n" tests
	For veterinary *in vitro* use only – For animal use only
	Keep away from sunlight

Notes

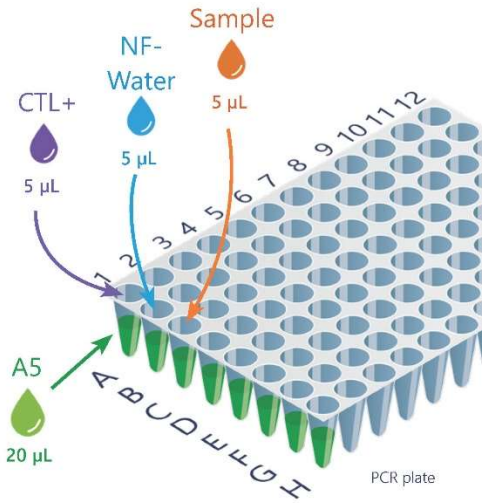
1 Extract nucleic acids with

Adia X Mag

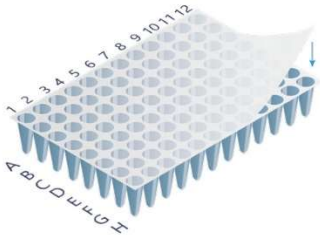
Scan me to discover Adiamag™

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

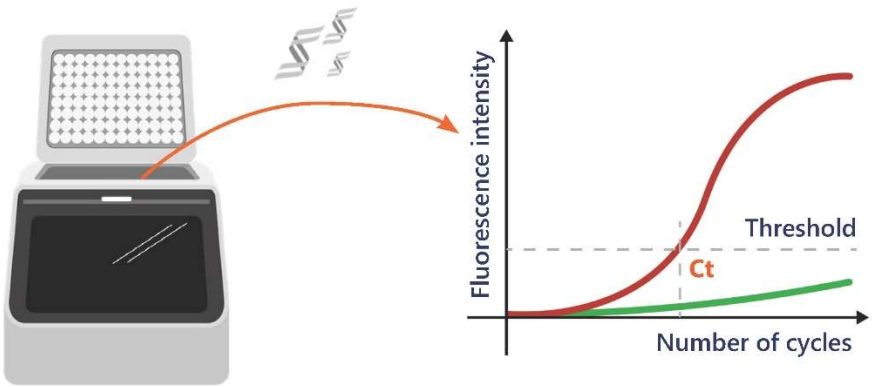
3 Distribute **5 µL** of nucleic acids, **CTL+** and **NF-Water**



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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