Instruction manual
ADL10Y1-BVDV_NO_(EN)_V03
09/2023

BVDV Triplex

Reference: ADL10Y1-100 & ADL10Y1-500

Test for the detection of Bovine Viral Diarrhea Virus by real time enzymatic amplification

PCR Test – 100 reactions & 500 reactions

For veterinary *in vitro* use only

Sample	Individual analysis	Pool of sample possible*, up to:
Blood/Serum	✓	20
Ear notch	✓	25
Tissue	✓	✗
Milk	✓	bulk

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

Kit composition

Content		ADL10Y1-100 Kit	ADL10Y1-500 Kit
		100 reactions	500 reactions
A6	Amplification solution	1 lyophilized vial with blank cap (To reconstitute	5 lyophilized vials with blank cap (To reconstitute)
Rehydration buffer	Rehydration solution	1 x 6 mL vial (Ready to use)	1 x 6 mL vial (Ready to use)
BVDV CTL+	BVDV positive control	1 tube with purple cap (To reconstitute)	2 tubes with purple cap (To reconstitute)
EPC-Ext	Exogenous extraction control	1 tube with yellow cap (To reconstitute)	3 tubes with yellow cap (To reconstitute)
NF-Water	Nuclease-Free Water	2 x 1000 µL tubes with white cap	4 x 1000 µL tubes with white cap (Ready to use)

Revision history

Date	Version	Modifications
01/2023	V01	Creation
07/2023	V02	Creation of new reference for 500R
09/2023	V03	Addition bulk milk matrix

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

A. Introduction

Bovine Viral Diarrhea Virus (BVDV), classical swine fever (CSFV) and border disease virus (BDV) in sheep are members of the pestivirus genus which belongs to the Flaviviridae family (like hepatitis C). BVDV, which induces mucosal disease in bovine, causes economic losses in cattle.

Many countries have started eradication programs of this disease, which involves a perfect management of infected animals. Indeed, those must be detected earlier with a high reliability. The earlier detection of these persistently infected animals is still necessary in eradication programs.

Most of these tests allow the detection of minimal quantities of BVDV in blood, serum, milk, or organs of infected animals, even with less than three months old animals.

B. Test principle

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

- BVDV, BDV and some CSFV (FAM labelled probe).
- Endogenous internal control of extraction and amplification (Endogenous) (HEX labelled probe or its equivalent).
- EPC-Ext: Internal control of extraction and amplification specific from an exogenous nucleic acid (Cy5 labelled probe).

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

- 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 BVDV EAR (Ref.: ADC10S02).** BVDV positive ear notch sample for extraction control.
- **Extraction Positive Control BVDV (Ref.: ADC10EPC).** Supplier reference material for method adoption that can also be used as extraction control.
- **LD_{PCR} Positive Control – BVDV (Ref.: ADC10YLD)** 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
ADIAMAG™ LB3 buffer	Buffer for magnetic beads	125 mL : ref. NADI004
ADIAPURE™ TLB	Direct lysis	400 tests : ref. ADIADP10E1-400

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.

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 allows to verify the reliability of the results. Controls must be included.

Control	Validation of	Usage
No Template Control (NTC)	Absence of amplification contamination	5 µL NF-Water in a well per run
BVDV CTL+	BVDV 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. A6 Amplification solution 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. It's used to reveal the presence of PCR inhibitors.

- 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.
- To use it, add **5 µL** of EPC-Ext in the first nucleic acids extraction lysis buffer.

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: Dispense **10 µL** of amplification solution (A6) 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: 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	538	554
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 (BVDV)	Cy5 (EPC-Ext)	HEX (Endo-genous)	
No Template Control (NTC)	No	No	No	Absence of amplification contamination
BVDV CTL+	Yes	No	Yes/No	Target amplification
Extraction negative control	No	Yes	No	Absence of extraction contamination
Extraction positive control	Yes	Yes	Yes/No	Extraction and amplification steps

2. Results interpretation

Amplification			Interpretation
FAM (BVDV)	Cy5 (EPC-Ext)	HEX (Endogenous)	BVDV/BDV
Yes	Yes	Yes	Detected
Yes	No	Yes	Detected
Yes	Yes	No	Detected
Yes	No	No	Detected
No	Yes	Yes	Undetected
No	No	Yes	Not determined[1]
No	Yes	No	Undetected for acellular matrix
			Not determined[2]
No	No	No	Not determined[3]

« Detected »: In the case of pool analysis, redo the analysis on individual samples of the pool to identify the animal positive for BVDV.
« Undetermined »: No characteristic amplification curve for critical controls.
Possible causes:
[1] Extraction issue and/or PCR inhibition.
[2] Sample forgotten or degraded during extraction.
[3] Potential PCR error/inhibition or error during the extraction.
Recommendations:
Set up a new PCR assay using pure nucleic acids extracts and 10x dilutions in Nuclease-free water or perform a new nucleic acids extraction.

Symbols

Symbole	Signification
REF	Catalog number
	Manufacturer
	Temperature limitation
	Use by
LOT	Batch code
	Consult Instructions for Use
Σ	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

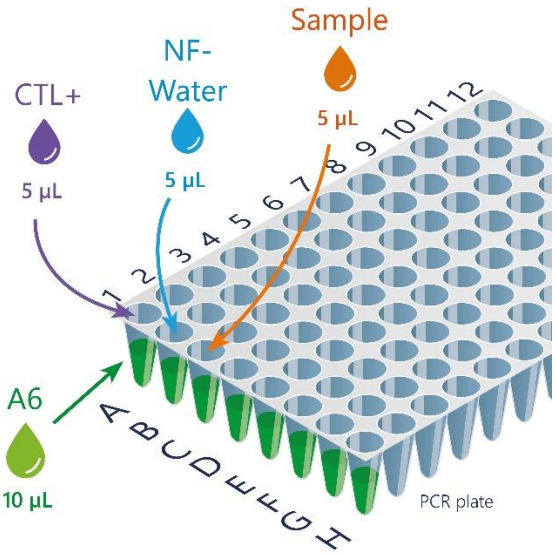
Adia X Mag →

Scan me to discover Adiamag™

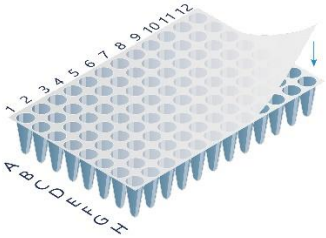
2 Add **1000 µL** of Rehydration buffer to the **A6** amplification solution

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

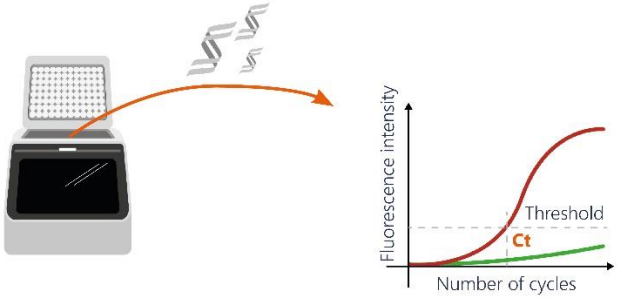
4 Distribute **5 µL** of nucleic acids, **CTL+** and **NF-Water**



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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