ADIAPURE™ TLB

DIRECTE LYSIS EXTRACTION KIT ON EAR NOTCH FOR THE DETECTION OF THE BOVINE VIRAL DIARRHOEA VIRUS BY REAL-TIME ENZYMATIC GENE AMPLIFICATION (RT-PCR TEST)

References:
ADIADP10E1-100 (100 extractions)
ADIADP10E1-400 (400 extractions)

English version
NE10E1-02
2020/11

ADIAPURE™ TLB

Revision history

N/A	Not Applicable (first publication)
Correction	Correction of document anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

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

Release Date	Part Number	Change type	Change summary
2020/07	NE10E1-01	N/A	First publication
2020/11	NE10E1-02	Technical change	Addition of 2 news extraction protocols (FAST LYSIS protocol and EASY LYSIS protocol)

I. General information

1. Purpose of the kit

ADIAPURE™ TLB is a nucleic acids RNA extraction kit without purification from ear notches. The nucleic acids obtained allows to detect the Bovine Viral Diarrhoea Virus (BVDV) and Border Disease Virus (BDV) with the PCR specific amplification kits of ADIAVET.

2. Description of test

The kit is based on directe lysis extraction of nucleic acids from ear notch for the detection of BVDV pathogens in combination with the ADIAVET™ range kits.

Analysis options according to the specimen:

Specimen	Individual analysis	Pool of sample is possible*, up to
Ear notch	☑	25

* It depends on the epidemiological case and on the quality of the specimen.

II. Material & reagents

1. Composition of kit

REF **ADIADP10E1-100**		
L1	Lysis buffer	1 x 30 ml (ready-to-use)
L2	Enzyme	2 x 1000 µl (ready-to-use)
REF **ADIADP10E1-400**		
L1	Lysis buffer	1 x 125 ml (ready-to-use)
L2	Enzyme	1 x 9 ml (ready-to-use)

* Contains a reagent at a concentration considered as dangerous: Proteinase K ,1,00%
Signal word: **DANGER**

H334
P261 / P280 / P342 + P311
Hazard statement(s):
H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Precautionary statement(s):
P261: Avoid breathing dust/fume/gas/mist/vapours/spray.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P342+P311: If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
Note:

A black diamond shape may appear on outer container labels. When located next to a danger symbol, read the danger symbol and the reference H and P number to hazard and precautionary statements. Otherwise, do not consider it as a symbol.

2. Validity and storage

On receipt, the kit should be stored at +2/8°C. For better stability, it is recommended to keep reagent L2 at a temperature below -15°C.
Do not mix reagents of two different batches.

3. Equipment required, but not supplied in the kit

Warning: The material should be Nuclease-free (e.g. autoclaved 25 minutes twice at +120°C or once 60 minutes at +121°C)
- Class II Microbiological Safety Cabinet
- Vortex
- 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 tubes of 5, 10 or 15 ml
- Powder-free latex or nitrile gloves
- Incubator, heating bath or block heater

Block heater specific of Allflex collector tubes, BVD HeatLyse System from Bio-X Diagnostics, including:
- Grant dry heating unit. Ref. NQBD2-EU
- Removable Allflex BVD heat block. Ref. N96Bloc05ML
- Allflex BVD 4*96 grippable sockets. Ref. N96TPACK

III. Use of the samples and the controls

1. Precautions

Before starting the process, read the entire protocol and scrupulously respect it.
We strongly recommend that only appropriately trained personnel perform this extraction. Ensure the accuracy and precision of the micropipettes used. The quality of the obtained results depends upon rigorous respect of good laboratory practices.

The PCR generates large amount of amplified DNA. A few molecules of amplified products are sufficient to generate a positive result. Do not open the PCR tubes after amplification.
Samples for analysis should be handled and disposed of as biological waste. **Take all measures of security and confinement required for the manipulation of the concerned biological agents.**

2. Storage of samples and nucleic acid extracts

Samples can be stored a couple of days at +2/8°C. With the exception of ear cartilage in the presence of desiccants which can be stored at 2/8 ° C for several weeks.
We recommend to store them at <-15°C after 2 days.
Extracted RNAs are quite sensitive molecules. Extraction is made at room temperature and should be performed as fast as possible to avoid degradations. Crude extracts should be stored at the end of extraction on melting ice or at +2/8°C for few hours, then at <-15°C.

3. Controls preparation

The use of controls allows verifying the reliability of the results.
The controls are included per trial of analysis. A trial is defined as all the samples treated in the same conditions.

All the steps of the analysis procedure (extraction+amplification), for all the types of samples, are validated with the association of the controls included in the kit.
The internal endogenous control (RNaseP) naturally found in the samples allows verifying the extraction and amplification steps of each sample.

Other controls must or could be added:

- Negative control of extraction (required)

To verify the absence of cross-contamination, at least one negative control must be included per trial (e.g. the normative requirement and recommendation for the development and the validation of veterinary PCR NF U47-600 suggests the use of 1 negative control for 24 samples or 4 negative samples for a 96 wells-plate). This control could be a negative matrix, or a buffer used for dilutions.

- Positive control of extraction (recommended)

A positive control could be added in each trial. The control is a sample including BVDV. It could come from a positive sample available in the laboratory or from a negative sample spiked with a solution of BVDV. This positive control will be closed to the limit of detection of the method. It will inform about the fidelity of the obtained results between different trials.

IV. Extraction protocols

Three extraction protocols are proposed for the lysis flash of ear notch with the ADIAPURE TLB:

<table>
<tr><th></th><th>CLASSIC LYSIS protocol</th><th>FAST LYSIS protocol</th><th>EASY LYSIS protocol</th></tr>
<tr><td>Preparation</td><td colspan="3">1 ear notch + 280 µl L1 TLB buffer + 20 µl L2 TLB buffer</td></tr>
<tr><td rowspan="2">Incubation</td><td>20 minutes at +65°C</td><td>8 minutes at +65°C</td><td rowspan="2">10 minutes at +95°C</td></tr>
<tr><td>15 minutes at +95°C</td><td>8 minutes at +95°C</td></tr>
<tr><td>Amplification</td><td colspan="3">5 µl RNA extracts used for the amplification with compatible PCR ADIAVET kit</td></tr>
</table>

1. Preparation of ear notch

Extract the ear tissue sample from the ear tag, e.g. in the collector tube.
Add

- **280 µl of L1 lysis buffet ADIAPURE™ TLB**
- **20 µl of L2 lysis buffer ADIAPURE™ TLB *.**

**A pre-mix of the both reagents could be prepared then 300 µl are added to each sample. The pre-mix is stable up to 5 days stored at +2/8°C.*

Seal and homogenize.

Carry out one of 3 options of incubations:

2. CLASSIC LYSIS protocol

Incubate

- **20 minutes** at **65°C**
- **15 minutes** at **95°C**
- **To ensure the accuracy of subsequent pipetting, allow the samples to cool** (e.g., 15-30 minutes at room temperature or 5-20 minutes at +2/4°C, according to the number of samples per assay)

NB1: Analysis on pooled samples is possible; mix in equal volume until 25 samples (e.g. 50 µl) and homogenize.

NB2: In case of a new analysis, each individual supernatant can be store at +2/8°C for 24 hours, then store them at <-15°C.

3. FAST LYSIS protocol

Incubate

- **8 minutes** at **65°C**
- **8 minutes** at **95°C**
- **To ensure the accuracy of subsequent pipetting, allow the samples to cool** (e.g., 15-30 minutes at room temperature or 5-20 minutes at +2/4°C, according to the number of samples per assay)

NB1: Analysis on pooled samples is possible; mix in equal volume until 25 samples (e.g. 50 µl) and homogenize.

NB2: In case of a new analysis, each individual supernatant can be store at +2/8°C for 24 hours, then store them at <-15°C.

4. EASY LYSIS protocol

Incubate

- **10 minutes** at **95°C**
- **To ensure the accuracy of subsequent pipetting, allow the samples to cool** (e.g., 15-30 minutes at room temperature or 5-20 minutes at +2/4°C, according to the number of samples per assay)

NB1: Analysis on pooled samples is possible; mix in equal volume until 25 samples (e.g. 50 µl) and homogenize.

NB2: In case of a new analysis, each individual supernatant can be store at +2/8°C for 24 hours, then store them at <-15°C.

V. Amplification

For the amplification of extracted nucleic acids, please refer to “Amplification” and “Interpretation of results” paragraphs of the user manual ADIAVET™ of the pathogen of interest.

VI. Index of symbols

Symbol	Meaning
	Catalogue number
	Manufacturer
	Upper temperature limit
	Use by date
	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Keep away from sunlight
	For veterinary in vitro use only

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