



## **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)**

**Reference:**

ADIADP10E1-400 (400 extractions)

# ADIAPURE™ TLB

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## Revision history

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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)
2022/09	NE10E1-03	Administrative	Remove of the ADIAPD10E1-100 reference Precision on the ear notch system compatible with the kit
2023/06	NE10E1-04	Technical change	Addition of 5 µL EPC-Ext in extraction step, according to the PCR kit used: ADIALYO BVDV ref. ADL10Y1-100
2023/06	NE10E1-04	Administrative	Addition of "Easy Prep BVD" ref. NIDC22PL-20, to facilitate test preparation.

## I. General information

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### 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.

### 2. Description of test

The kit is based on direct lysis extraction of nucleic acids from ear notch for the detection, without purification, of the BVDV virus.

Analysis options according to the specimen:

Specimen	Individual analysis	Pool of sample is possible*, up to
Ear notch	<input checked="" type="checkbox"/>	25

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

TST and TST-L systems of Allflex and 901372 FlexoPlus PP geno dry preservative system of Caisley are compatibles with this kit. Other systems must be evaluated before use.

## II. Material & reagents

### 1. Composition of kit

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 TST Allflex collector tubes, BVD HeatLyse System from Bio-X Diagnostics, including:**

- Easy Prep BVD. Ref. NIDC22PL-20
- Grant dry heating unit. Ref. NQBD2-EU
- Removable Allflex BVD heat block. Ref. N96Bloc05ML
- Allflex BVD 4\*96 grippable sockets. Ref. N96TPACK

For block heater specific of TST-L Allflex collector tubes, please contact your Bio-X Diagnostics commercial department or [support.pcr@biox.com](mailto:support.pcr@biox.com).

### III. Use of the samples and the controls

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#### 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.

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 storing 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. 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

Extract the ear tissue sample from the ear tag, e.g. in the collector tube.  
In the case of an ALLFLEX TST-L system, remove the conservation fluid from the collection tube.

Add to ear notch:

- **280 µL of L1 lysis buffer ADIAPURE™ TLB**
- **20 µL of L2 lysis buffer ADIAPURE™ TLB**
- **5 µL of EPC-Ext (according to the PCR kit used)**

*\*A pre-mix could be prepared, then 305 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:

	<b>CLASSIC LYSIS protocol</b>	<b>FAST LYSIS protocol</b>	<b>EASY LYSIS protocol</b>
<b>Preparation</b>	1 ear notch + 280 µL L1 TLB buffer + 20 µL L2 TLB buffer + 5 µL EPC-Ext (according to the PCR kit used)*		
<b>Incubation</b>	20 minutes at +65°C	8 minutes at +65°C	10 minutes at +95°C
	15 minutes at +95°C	8 minutes at +95°C	
<b>Preparation of pooled samples</b>	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)  Analysis on pooled samples is possible until 25 samples** by mixing in equal volume (e.g. 50 µL) and homogenize		
<b>Amplification</b>	5 µL RNA individual or pool extracts are used for the amplification with compatible PCR kits		

*\* The EPC-Ext provided in the kit of the pathogen of interest. Refer to the corresponding package insert for preparation, storage and use of the control.*

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

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

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

## VI. Index of symbols

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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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