PRODUCT DESCRIPTION

Flash Lysis Buffer
Without nucleic acids purification with Tissue Lysis Flash (TLF)

Cat N° TLF10ml
Cat N° TLF50ml
Cat N° TLF100ml

For the use with Bio-T kit® BVDV/BDV Universal
From individual ear notches
or from pool up to 10
and from individual serum or from pool up to 20

Sample types

- Individual ear notches or pool up to 10
- Individual serum or pool up to 20

Key points

- 5 minutes of Flash Lysis at 95°C ± 1.5 in TLF buffer
- Direct use of the lysate at RT-PCR
- Protocol is validated only for use with Bio-T kit® BVDV/BDV Universal from ear notches and from serum

Veterinary use only

DOCUMENTS MANAGEMENT

The Bio-T kit® BVDV/BDV Universal has two technical handbooks:

- The extraction handbook shared between the Bio-T kit® BVDV/BDV Universal and Bio-T kit® BVDV Genotyping, displaying BioSellal's validated or recommended extraction protocols for each type of sample.
- The Bio-T kit® BVDV/BDV Universal qRT-PCR handbook, presenting the instruction information to perform the qRT-PCR.

The last versions in use for each handbook are indicated on the certificate of analysis (CA) provided with the Bio-T kit® BVDV/BDV Universal.

Besides these two handbooks, the TLF handbook describes the composition of the product as well as the key points of its handling.

MODIFICATIONS MANAGEMENT

BioSellal indicates modifications done to this document by highlighting them using the rules presented in the Table below:

MODIFICATIONS MANAGEMENT			
Type of modification Highlighting color	**Minor modifications**	**Type 1 Major modifications**	**Type 2 Major modifications**
Impact on revision / version	**Change of revision date** **No change of version**	**Change of revision date** **+ change of version**	**Change of revision date** **+ change of version**
Examples of modifications	Corrections: typographical, grammatical or turns of phrase	EPC reference modification	Modification of Master Mix composition
	Addition of new sample type for extraction	Exogenous IPC reference modification	Modification of validated extraction protocol
	Addition of information giving more details or alternative protocol		
	Addition/Suppression of optional information		

PRESENTATION

TLF Buffer description

TLF buffer is a lysis solution for extra fast samples preparation without nucleic acids purification.

The protocol is adjusted and validated by BioSellal (volume and holding time optimization) and reserved for the detection of BVD/BD viruses from ear notches (individual or mix up to 10) and from serum (individual or mix up to 20) with the Bio-T kit® BVDV/BDV Universal.

One bulk of TLF10ml enables to perform 100 individual lysis (One TLF tube of 10 ml). One bulk of TLF50ml enables to perform 500 individual lysis (5 TLF tubes of 10 ml) and one bulk of TLF100ml enables to perform 1000 individual lysis (1 TLF bottle of 100 ml). **ATTENTION, TLF100ml thawing takes about 4 hours at room temperature**. It is recommended that TLF should be aliquoted in small volumes for one run of extraction. Keep TLF, at ≤ -16°C till the expiration date indicated at the label.

When using, defrost the TLF extemporaneously and store at 5 ° C ± 3. Indeed, keeping TLF at room temperature can affect its performances. Thus, it is very important to minimize the duration time at room temperature to ensure optimal performance. After use, refreeze the rest of TLF at ≤ -16°C. It's recommended not to exceed more than 3 freezing thawing cycles of one tube.

List of consumables and reagents not included

For serum samples, **it is mandatory to use Exogenous IC** (ICBVDU-A **tube with purple cap**), **supplied in Bio-T kit® BVDV/BDV Universal. It has to be used during the extraction step for each sample and extraction controls (NCS, MRI)**. BioSellal recommends using also the exogenous IC for ear notches in order to validate the absence of PCR inhibitors.

Table 1. Consumables and reagents not included in kit

Consumables/ Reagents	Description		Provider	Cat. N°
BioExtract® Column	DNA/RNA column extraction kit	(50)	BioSellal	BEC050
		(250)		BEC250
BioExtract® SuperBall®	DNA/RNA Magnetic beads extraction kit (4 x 96)			BES384
Cador® Pathogen 96 QIAcube® HT kit	DNA/RNA extraction kit Plate format (x5)		Indical	SP54161
TLF Buffer	Flash Lysis Buffer	1 tube of 10 ml	BioSellal	TLF10ml
		5 tubes of 10 ml		TLF50ml
		1 bottle of 100ml		TLF100ml
Flash Lysis Heat block for ear notches	Heat block adapted for Allflex tubes		BioSellal	BTLF
Flash Lysis Heat block for serum	Heat block adapted for 96-well plate		Supplier of your choice	
Metallic foil seal	96-well Plate Foil Seal		Agilent Technologies	5067-5154
PBS Buffer¥	Phosphate Buffered Saline 1X		Supplier of your choice¥	

For consumables related to the thermal cycler, refer to the user manual of the device.
¥ The use of molecular biology grade PBS is very important to obtain optimal results for sample extraction with protocols validated by BioSellal (Flash Lysis, BioExtract® Superball ® and BioExtract® Column). Thus, BioSellal recommends using a commercial PBS with a Molecular Biology grade quality.

Main critical points

- Wear appropriate personal protective equipment (lab coat, disposable gloves frequently changed).
- Work in dedicated and separate areas to avoid contamination: "Extraction" (unextracted samples storage, extraction equipment area), "Mix" (ready to use MM storage, qRT-PCR plates preparation), "Nucleic acids Addition" (Nucleic Acids storage and addition of extracted nucleic acids and controls in the qRT-PCR plate), "PCR" (final area containing the thermal cycler(s)).
- Use dedicated equipment for each working area (gloves, lab coat, pipettes, vortex...).
- Use filter tips.
- Before use, thaw all components at room temperature.
- Vortex and spin briefly (mini-centrifuge) all reagents before use.
- Avoid the repetition of freezing-thawing cycles for samples, lysates, extracted nucleic acids.
- **Genomes of pathogens detected by the RUMINANTS line kits can be DNA or RNA. Working with RNA is more demanding than working with DNA** (RNA instability and omnipresence of the RNases). Genomes of Ruminants line are DNA or RNA. For these reasons, special precautions must be taken:
    - Always wear gloves, change them frequently, especially after contact with skin or work surfaces.
    - Treat all surfaces and equipment with RNases inactivation agents (available commercially).
    - When wearing gloves and after material decontamination, minimize the contact with surfaces and equipment to avoid the reintroduction of RNases.
    - Use “RNase free” consumable.
    - It is recommended to store the RNA at ≤ 5°C ± 3 during the manipulation and then freeze it as soon as possible, preferably at ≤ -65°C or by default at ≤ -16°C.
    - Open and close tubes one by one to limit the opening times and avoid any contact with RNases present in the environment (skin, dust, working surfaces…).

At theses general precautions, on organs from aborted fetus, precautions related to the zoonotic risk are added, due to possible presence of *Coxiella burnetii*, *Listeria*, *Leptospira*, *Salmonella…*

- When samples are received, it is necessary to disinfect the containers with the help of sporocide before or/and handling under the MSC.
- During the extraction it is mandatory to handle under the MSC till the end of sample lysis, because of the zoonotic risk associated with the manipulated sample types and the pathogenic agent presence.

VALIDATED AND RECOMMENDED NUCLEIC ACIDS EXTRACTION METHODS

GENERALITY

The characterization of the complete method associating serum and ear notches extraction with the qRT-PCR kit (Bio-T kit® BVDV/BDV Universal° was developed and validated according to the **French regulatory standard NF U47-600-2 edited by AFNOR** and the specification of **the French Expert Laboratory for the BVD (ANSES-Niort).**

Recommendations for sampling, shipping and storage of samples

Real-time qPCR is a powerful technique allowing the detection of few amounts of pathogen genome. Genome can be rapidly degraded depending on the pathogen nature (bacteria, parasites, enveloped viruses..), the genome nature (DNA / RNA) and the sample type (presence of DNase / RNase). Thus, BioSellal recommends the following instructions to guarantee an optimal diagnosis.

Sampling

- Individual ear notches or pool up to 10
- Individual serum or pool up to 20

To prevent cross-contamination between samples leading to false positive results, it is mandatory to use disposable materials for single use and to avoid direct contact between specimens.

Shipping

It is mandatory to ship within 7 days after collection and to conserve under cover of the positive cold before shipping of ear notches.

Storage after reception

It is recommended to immediately analyze samples after receipt or freezing at ≤ -16 ° C for a few months and ≤ -65°C beyond 1 year.

FLASH LYSIS EXTRACTION PROTOCOL

For sample pooling and processing protocol for Flash Lysis, refer to the Bio-T kit® BVDV / BDV Universal extraction handbook. The different steps for qRT-PCR are detailed in the Bio-T kit® BVDV / BDV Universal qRT-PCR handbook.

Note: BioSellal recommends using nucleic acids obtained after flash lysis buffer within 30 minutes after extraction.

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