Certified management system

Direct lysis buffer

100 / 500 preps

Product code: DLB

Direct lysis extraction buffer for the detection of Bovine Viral Diarrhea virus

Suitable samples: Ear notch Individual samples or pools of up to 25

***In vitro* use**

March 2018 : Modification of the instruction for use
- Modification of the incubation temperature with EZNOTCH-PLATE

0997

IDvet Genetics, 310, rue Louis Pasteur – Grabels - FRANCE
Tel:+ 33 (0)4 67 41 49 33 - Fax: + 33 (0)4 67 45 36 95
www.id-vet.com - E-mail: info@id-vet.com

General information

- **Characteristics**

The Direct Lysis Buffer (DLB) is a direct lysis extraction buffer of ear notch samples for the detection of Bovine Viral Diarrhea virus (BVDV). This buffer can be used to lyse individual ear notch samples or pools (of up to 25).

It is recommended to combine the DLB with the ID Gene™ BVD/BD Triplex (IDBVD) kit (which includes controls) for the amplification step.

- **DLB storage conditions**

Reference	*Component*	*Volume*	*Description*
DLB	Direct Lysis Buffer	77 ml 1 bottle	Ready-to-use Direct lysis buffer

The solution should be stored at ≤ -16°. It is recommended to prepare aliquots in order to avoid multiple freeze/thaw cycles (> 3 not recommended).

- **Required equipment, consumables and reagents not provided in the kit**

All material used should be of suitable quality for molecular biology.

Equipment and consumables:

- Precision pipettes capable of delivering volumes of 1 µl to 1000 µl
- Nuclease-free filter tips
- Sample tubes for ear notch samples (*e.g.* EZNOTCH-TUBES, 1.5ml or 2 ml tubes)
- System for heating sample tubes: IDvet Genetics proposes racks and caps adapted (see table below). Contact support.genetics@id-vet.com for more information
- Heating block (*e.g.* Eppendorf Thermomixer®)
- 0.5 ml tubes, 1.5 ml tubes or micro-plates for RNA storage

Reagents:

- Distilled or Nuclease-free water
- ID Gene™ BVD/BD Triplex (IDBVD)

Optional Consumables not supplied with the kit :

Consumables	*References*	*Description*
Eppendorf ThermoMixer C® 	Contact support.genetics@id-vet.com	Heating block with plate or tubes adapter (provided with heated lid), capable of heating at 100°C while shaking (500 rpm)
EZNOTCH-PLATE	655201	EZNOTCH-TUBES rack
EZNOTCH-TUBES 	62503036	Tubes for ear notch samples (type : Allflex® TST dry), Pack of 100
EZNOTCH-CAPS 	038791	Strip of 8 caps for EZNOTCH-TUBES

Remarks and precautions

The material used contains less than 0.1% hazardous or carcinogenic substances, thus MSDS sheets are not required. However, it is recommended to take appropriate precautions, as with any biochemical product, and to wear appropriate clothing.

Direct Lysis Protocol

1. **Prepare samples and controls (provided in the ID Gene™ BVD/BD Triplex kit (IDBVD) as described below:**

Reagent	*Sample*	*Controls*	
		Positive	*Negative*
Direct Lysis Buffer (DLB)	140 µl	140 µl	
Sample or Control	**Ear notch sample**	**50 µl TPC-EN-BVD**	**50 µl of negative ear notch sample**
NTPC-EN-BVD	**20 µl**	**20 µl**	

2. **Lysate samples and controls following these 2 incubation steps:**

	Tubes	*EZNOTCH-PLATE*
Short incubation	Heat at **70°C for 30 min** then at **90°C for 10 min**	Heat at **70°C for 30 min while shaking** (500 rpm) then at **100°C* for 15 min while shaking** (500 rpm)
Overnight incubation	Incubate at **21°C (± 5°C)** for **16h to 20h** then **90°C for 10 min**	Incubate at **21°C (± 5°C) for 16h to 20h** then at **100°C*** for **15 min while shaking** (500 rpm)

* set point temperature and adapted incubation time for specific lyse in « EZNOTCH-PLATE », these specific temperature and time are required to reach the target temperatures for optimum activity.

3. **At the end of lysis steps:**
   a. Transfer **100 µl** of each sample or control lysate, into a labelled micro-tube or micro-plate.
   b. Keep the samples at 5°C (± 3°C) for at least 5 minutes

If testing pooled samples:

a. Perform lysis for each individual sample.

b. Pipette **20 µl** of each individual lysate and pool them into the same tube (pools of up to 25).
In the case of a pool with less than 25 samples, take an equivalent volume of each individual lysate in order to obtain a final volume of 500 µl.

c. Homogenize the pool by vortexing.

4. **Keep the lysates at 5°C (± 3°C) if the PCR is to be performed immediately. Store at <-16°C for long-term conservation.**
5. **Proceed with the amplification reaction of the lysates using the ID Gene™ BVD/BD Triplex (IDBVD).**

Documentation and support

For questions or technical support, please contact: support.genetics@id-vet.com

For additional information, visit www.id-vet.com

Certified management system

Direct lysis buffer

100 / 500 preps

Product code: DLB

Direct lysis extraction buffer for the detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae*

Suitable samples: tracheal or oropharyngeal swabs, FTA® cards samples (individual samples or pool of up to 3).

***In vitro* use**

IDvet Genetics, 310, rue Louis Pasteur – Grabels - FRANCE
Tel:+ 33 (0)4 67 41 49 33 - Fax: + 33 (0)4 67 45 36 95
www.id-vet.com - E-mail: info@id-vet.com

General information

- **Characteristics**

The Direct Lysis Buffer (DLB) is a direct lysis extraction buffer for tracheal or oropharyngeal swabs and FTA® cards samples for the detection of *Mycoplasma gallisepticum (MG)* and *Mycoplasma synoviae (MS)*. This buffer can be used to lyse individual tracheal or oropharyngeal swabs or pools (of up to 3).

It is recommended to combine the DLB with the ID Gene™ MG/MS Triplex (IDMGMS) kit (which includes controls) for the amplification step.

- **DLB storage conditions**

Reference	*Component*	*Volume*	*Description*
DLB	Direct Lysis Buffer	77 ml 1 bottle	Ready-to-use Direct lysis buffer

The solution should be stored at ≤ -16°. It is recommended to prepare aliquots (minimum 100 µl) in order to avoid multiple freeze/thaw cycles (> 3 not recommended).

- **Required equipment, consumables and reagents not provided in the kit**

All material used should be of suitable quality for molecular biology.

Equipment and consumables:

- Vortex
- Nuclease-free tubes of 2 ml and 15 ml
- Precision pipettes capable of delivering volumes of 1 µl to 1000 µl
- Nuclease-free filter tips
- System for heating sample tubes: IDvet Genetics proposes racks and caps adapted for heating tubes. Contact info@id-vet.com for more information
- Heating block (*e.g.* Thermomixer®) capable of heating to 90°C with plate adapter or compatible tubes
- 0.5 ml tubes, 1.5 ml tubes or micro-plates for DNA storage

Reagents:

- 1X PBS or TE buffer (10mM tris- 1mM EDTA) (molecular biology quality)
- Distilled or Nuclease-free water
- ID Gene™ MG/MS Triplex (IDMGMS)

Remarks and precautions

The material used contains less than 0.1% hazardous or carcinogenic substances, thus MSDS sheets are not required. However, it is recommended to take appropriate precautions, as with any biochemical product, and to wear appropriate clothing.

Direct Lysis Protocol

1. Samples preparation

For tracheal or oropharyngeal swabs

Note: It is recommended to treat individually heavily soiled swabs.

- Dispense **1ml of 1X PBS or TE buffer** into a 15 ml tube (or hemolysis tube or equivalent)
- **Cut off** by scissors or snapped off **at the break point 1 to 3 swabs**.
- Place the swabs in the 15 ml tube.
- Vortex for 30 seconds.
- **Collect 50 µl** of **the supernatant** for the extraction, store the volume left at -20°C.

For FTA® cards

For individual samples :

- Collect **3 sample discs** from each sample spot using a coring device (a 1.6 to 2 mm disc is recommended).

For Pooled samples (**up to 3 samples**)

- Collect **1 sample disc** from each sample spot using a coring device (a 1.6 to 2 mm disc is recommended) up to **3 discs** to be collected (1 disc per sample).

- **Place the discs** in a tube containing **1ml of 1X PBS or TE buffer**
- Vortex for 30 seconds.
- **Collect 50 µl** of the supernatant for the extraction, store the volume left at -20°C.

2. Controls preparation (provided with the IDMGMS kit)

The negative and positive controls should be prepared and extracted at the same time as the samples being tested.

- **Add 550 µl** of Nuclease-free water to the freeze-dried **TPC-MGMS**. 50 µl will be necessary for the extraction.
- For the NEC (Negative extraction control) :
  - If the NEC is prepared with a sample of known negative status, follow the classic method of pre-treatment.
  - If the NEC is prepared with water, follow this procedure with 50 µl of Nuclease-free water.

3. Addition of the direct Lysis Buffer

- **Pipette 50 µl** of **the supernatant** from swabs or FTA® cards, **TPC-MGMS** and negative matrix or water (**NEC**) in nuclease-free tubes of 2 ml.
- Add **140 µl** of **DLB.**

4. Incubation steps

- Incubate tubes for **30 minutes at 70°C.**
- **Immediately** incubate tubes, without cooling, for **10 minutes at 90°C.**
- Transfer **100 µl** of each sample or control lysate, into a labelled micro-tube or micro-plate.

5. Keep the lysates at 5°C (± 3°C) if the PCR is to be performed immediately.

Maintain at <-16°C for long-term storage.

6. Perform the amplification reaction of the lysates with ID Gene™ MG/MS Triplex (IDMGMS).

Documentation and support

For questions or technical support, please contact: info@id-vet.com

For additional information, visit www.id-vet.com