Instruction manual
ADPLF1_FLEX_NO_(EN)_V01
01/2025

AdiaX Pure

ADIAPURE LYSIS FLEX

Reference: ADPLF1-500

Direct lysis extraction kit for the detection of nucleic acids by real-time enzymatic gene amplification (PCR test) use with ADIALYO™ PCR range.

Extraction kit – 500 mL

For veterinary *in vitro* use only

Kit composition

Content		ADPLF1-500 Kit
		500 mL
LF1	Lysis Buffer	5 x 100 mL
L2	Proteinase K	1 x 1.1 mL
LF3	Lysis Buffer	1 x 28 mL

Revision history

Date	Version	Modifications
03/2024	NELF1-04	Addition of *Mycobacterium avium* subsp. *paratuberculosis* protocol from faeces
01/2025	V01	New layout Addition of *Ornithobacterium rhinotracheale* protocols. Addition of Africain Swine Fever Virus, *Lawsonia intracellularis*, *Brachyspira spp*, *Actinobacillus pleuropneumoniae* and *Mycoplasma hyopneumoniae* protocols.

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

Validated protocols

Pathogen	FTA card §F	Swab §G.1.a
Mycoplasma gallisepticum	X	X
Mycoplasma synoviae		
Mycoplasma meleagridis		
Mycoplasma iowae		
Ornithobacterium rhinotracheale		
Bronchitis Infectious Virus		
Influenza Virus		

Pathogen	Blood on			Serum	Tissue			Faeces			Drag swab	Swab/brush	Tracheobronchial fluid	FTA card
	EDTA	Swab	Whatman 3 paper		Grinding	Swab	Biopsy	1g faeces	Swab	ADIAPREP				
	§G.1.b			§G.1 .c	§G.1.d			§G.1.g			§G.1.f	§G.1.a	§G.1.e	§F.
Influenza Virus												X		
Lawsonia intracellularis					X	X		X	X	X	X	X		
Brachyspira spp.					X	X		X	X	X	X	X		
Mycoplasma hyopneumoniae					X	X						X	X	X
Actinobacillus pleuropneumoniae							X					X		
Africain Swine Fever Virus (ASFV)	X	X	X	X	X									

Pathogen	Faeces ADIAPREP §G.1.g
Mycobacterium avium subsp. *paratuberculosis*	X

A. Test principle

The ADIAPURE™ LYSIS FLEX extraction kit allows nucleic acid extracts to be obtained by direct lysis. It contains all the buffers required for nucleic acids extraction from different matrices. The obtained nucleic acids can then be used without purification for pathogen detection by direct amplification with kits belonging to the ADIALYO™ range.

B. Storage conditions

At reception, the kit should be aliquoted and stored at +2/8 °C. For better stability, it is recommended to keep L2 reagent at a temperature below -15 °C. In this case, the kit is stable at least 1 year. Do not mix reagents of two different batches.

C. Material required but not provided

- Class II Microbiological Safety Cabinet.
- Centrifuge for microtubes.
- Grinder (Mixer Mill or Fast Prep).
- Incubator type Thermocycler or blocks heater.
- 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.
- PCR plates and aluminium foil (for heating in the thermocycler).
- Sterile tubes of 5, 10 or 15 mL.
- Razor blades.
- Sterile saline water (NaCl 8.5 g/L).
- 1X PBS buffer.
- Powder-free latex or nitrile gloves.
- Fecal preparation system ADIAPREP™ (Bio-X Diagnostics ref.: 200 tests, ADPREP-200).
- Metal beads 3 mm for grinding with Mixer Mill.
- Glass beads for grinding with Mixer Mill:
  - ADIAPURE™ ALIQUOTED GLASS BEADS (Bio-X Diagnostics, 480 tests: ref. ADIADPBIA-480).
  - ADIAPURE™ GLASS BEADS RACKS 4x96 (Bio-X Diagnostics, 384 tests: ref ADPBIAR-4x96).

D. 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).

E. Controls to include

Using controls allow to verify the reliability of the results. Controls can be included.

Control	Validation of	Usage
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

F. Nucleic acids extraction from FTA card

FTA card protocol
Cut a 3 mm^2 piece of the FTA Card.
Place it in a microtube.
Add **100 µL of LF1 buffer**, vortex 10 sec and discard the liquid.
Add **100 µL of LF3 buffer**, vortex 10 sec and discard the liquid.
Add **100 µL of LF3 buffer**, vortex 10 sec and incubate **5 minutes** at **+95 °C**.
Allow cooling to ensure the accuracy of subsequent pipetting.
*Note: add **0.5 µL of EPC-Ext/PCR well** during the amplification stage for the kits concerned.*

G. Nucleic acids extraction from animals' matrices

1. Sample preparation

a. From swab

Avian swabs

- Cut **1 to 6 swabs** in a 5 mL tube.
- Add **1 mL of LF1 buffer** in the case of 1 to 3 swabs analysis or add **2 mL of LF1 buffer** in the case of 4 to 6 swabs analysis.
- Mix.
- Use **50 µL of the supernatant** for the next step §" Sample lysis".

Swine swabs/brushs

- Cut **1 to 3 swabs** in a 5 mL tube.
- Add **1 mL of LF1 buffer** and mix.
- Use **50 µL of the supernatant** for the next step §" Sample lysis".

b. From blood

EDTA Blood

- Transfer **100 µL of blood** in **400 µL of LF1 buffer** and mix.
- Use **50 µL of the supernatant** for the next step §" Sample lysis".

Swab blood

- Cut **the blood swab** in a 5 mL tube.
- Add **1 mL of LF1 buffer** and mix.
- Use **50 µL of the supernatant** for the next step §" Sample lysis".

Blood on Whatman3 blotting paper

- Cut **5 pieces** in a 5 mL tube and add **1 mL of LF1 buffer**
- Mix and incubate 10 to 15 minutes at room temperature.
- Use **50 µL of the supernatant** for the next step §" Sample lysis".

c. From serum

- Use **50 µL of serum** for the next step §" Sample lysis".

d. From tissue

Grinding protocol	Swab of tissue protocol
Transfer **20 to 100 mg** of tissue in a tube. Add **1 mL of LF1 buffer**. Grind (for example, using the Mixer Mill: add 1 metal bead, grind 2 minutes at 30Hz). Centrifuge 6 000g/2 minutes. Use **50 µL of suspension** for the next step §" Sample lysis".	Rub a dry swab on tissue. Cut the swab in 1 mL of LF1 buffer and mix. Use **50 µL of suspension** for the next step §" Sample lysis".

Biopsy protocol
Transfer a **biopsy** in **1 mL of LF1 buffer** and mix. Use **50 µL of suspension** for the next step §" Sample lysis".

e. From tracheobronchial fluid

- Centrifuge 1 mL of tracheobronchial fluid, 30 minutes at 10 000 g.
- Remove the supernatant, then add **200 µL of LF1 buffer** and mix.
- Use **50 µL of the supernatant** for the next step §" Sample lysis".

f. From environmental sample (drag swab)

- Add 40 mL of PBS 1x or sterile saline water in the drag swab. Knead.
- Centrifuge 1 mL of suspension 10 minutes at 1 000 g.
- Use **50 µL of the supernatant** for the next step §" Sample lysis".

g. From Faeces

1 g of faeces protocol	Faecal swab protocol
Weigh out **1 g faeces** and transfer to 5 mL PBS 1x or saline water. Mix. Use **50 µL of suspension** for the next step §" Sample lysis".	Dip a dry swab into the faeces jar. Cut the swab in **1 mL of LF1 buffer** and mix. Use **50 µL of suspension** for the next step §" Sample lysis".

ADIAPREP protocol
▪ Collect 1 spoonful of faecal matter using the ADIAPREP device. Scrape off the spoonful and reinsert it into the device. Vortex until a homogeneous suspension is obtained. ▪ Transfer 1 mL into a microtube or 96-well plate. ▪ Centrifuge for 5 minutes at 3 000 g and remove the supernatant with a pipette. ▪ Add 300 mg of glass beads (for example, ADIAPURE ALIQUOTED GLASS BEADS) and 500 µL of LF1 buffer to the pellet. ▪ Grind for 5 minutes at 30 Hz on Mixer Mill or 3 x 45 seconds at 4 m/sec on Fast Prep/Ribolyser then centrifuge for 5 minutes at 3000 g. ▪ Use **50 µL of suspension** for the next step §" Sample lysis".

2. Sample Lysis

For each sample:

- Prepare 50 µL of **LF3 buffer** + 2 µL of **L2 buffer**, **and 5 µL of EPC-Ext** for the kits concerned, in a microtube or PCR well.

A pre-mix containing the reagents can be prepared and distributed for each sample at the same time.

- Add **50 µL of prepared sample**.
- Close and mix.
- Incubate 5 minutes at +65 °C, then 15 minutes at +95 °C in a suitable heat block or thermocycler.
- Allow samples to cool to ensure accuracy of subsequent pipetting.
- Use 5 µL of extracts for PCR amplification (see § "Amplification").

H. Conservation of nucleic acids

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.

I. Amplification

For the amplification of extracted nucleic acids, please refer to "Amplification Protocol" and "Reading and Interpretation" paragraphs of the ADIALYO™ user manual of the pathogen of interest.

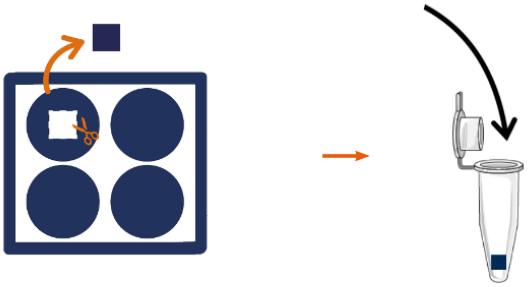
Symbols

Symbol	Signification
	Catalog number
	Manufacturer
	Temperature limitation
	Use by
	Batch code
	Consult Instructions for Use
	Contain sufficient for "n" tests
	For veterinary *in vitro* use only – For animal use only
	Keep away from sunlight
	Keep dry

Notes

Extraction from FTA card

1 Cut a **3 mm²** piece of the **FTA card**.
Place it in a microtube.



2 Add **100 µL** of **LF1** buffer,
vortex **10 sec** and discard the liquid

3 Add **100 µL** of **LF3** buffer,
vortex **10 sec** and discard the liquid

4 Add **100 µL** of **LF3** buffer,
vortex **10 sec** and
incubate **5 minutes** at **95°C**

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