ADIAPURE™ Lysis FLEX

DIRECT LYSIS EXTRACTION KIT
FOR THE DETECTION OF NUCLEIC ACIDS
BY REAL-TIME ENZYMATIC GENE AMPLIFICATION (PCR TEST)

USE WITH ADIALYO RANGE ONLY

References:
ADPLF1-500

VET

English version
NELF1-04
2024/03

ADIAPURE™ Lysis FLEX

Main change since previous version

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
2020/05	NELF1-01	N/A	First publication
2023/06	NELF1-02	Technical	Addition of Influenza virus and Infectious Bronchitis Virus detection from swabs
2023/10	NELF1-03	Technical	Addition protocol from FTA CARD
2024/03	NELF1-04	Technical	Addition of *Mycobacterium avium* subsp. *paratuberculosis* detection from faeces

I. General information

1. Purpose of the kit

ADIAPURE™ Lysis FLEX is a nucleic acids extraction kit based on chemical lysis. The nucleic acids can be used without purification with the PCR amplification kit of **ADIALYO**™ range validated with the kit.

2. Description of test

Adiagene validated the ADIAPURE™ Lysis FLEX kit from swabs, FTA cards, faeces, and environmental samples for the detection of pathogens in combination with the **ADIALYO**™ range.
The following table summarises the validated protocols.

		Swabs	FTA CARDs	Faeces	Environmental samples (dung scraping)
Avian diseases	*Mycoplasma gallisepticum*	X	X		
	Mycoplasma synoviae	X	X		
	Mycoplasma meleagridis	X	X		
	Mycoplasma iowae	X	X		
	Influenza Virus	X	X		
	Infectious Bronchitis Virus	X	X		
Ruminants diseases	*Mycobacterium avium subsp. Paratuberculosis*			X	X

For the pool size, refer to the user manual of the ADIALYO™ kit concerned.

II. Material & reagents

1. Composition of kit

The ADIAPURE Lysis FLEX kit contains the following buffers:

REF ADIADP01S1-500		
LF1	Lysis buffer	5 x 100 mL (ready-to-use)
L2	Enzyme	1 x 1.1 mL (ready-to-use)
LF3	Lysis buffer	1 x 28 mL (ready-to-use)

2. Validity and storage

On receipt, 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.

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.
- Incubator, heating bath or block 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.
- Sterile tubes of 5, 10 or 15 mL.
- Powder-free latex or nitrile gloves.
- Fecal preparation system:
    - ADIAPREP™ (Bio-X Diagnostics ref.: 200 tests, ADPREP-200).
- Grinding beads.

  For 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).

  For Rybolyser and Fast prep:
    - Lysing Matrix B (MP biomedicals, 100 tubes, ref. 116911100).

III. Use of the samples and the controls

1. Precautions

Caution:
Prepare the buffers of the kit according to the §II.2.
The buffers could contain toxic substances, please consult the MSDS safety data sheet.
The storage temperature must be respected.

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.**

Before starting the process, read the entire protocol and scrupulously respect it.

2. Storage of nucleic acid extracts

Extracted nucleic acids are temperature sensitive molecules. Crude extracts should be stored at the end of extraction on melting ice or at +2/8 °C for up to 24 hours, then at <-15 °C.

3. Controls preparation

Several controls should be included per trial of analysis.
The use of different controls included in the ADIALYO™ kits allows validating all the analysis process steps (extraction and amplification) for all the samples.

- The endogenous or exogenous internal control included in the ADIALYO™ kits allow validation of the extraction and amplification steps for every sample.
- The positive control included in the ADIALYO™ kits allows validation of the amplification of the specific target.

Other controls should or must be added:

A. Negative control of extraction (required)

To verify the absence of cross-contamination, at least one negative control must be included per trial (e.g. AFNOR NF U47-600-1 guidelines suggest including a negative control per 24 columns centrifuged or four negative controls per trial of 96-wells plate). The control is a negative sample, for example a buffer used for dilution.

B. Positive control of extraction (recommended)

A positive control could be added in each trial. The control is a sample including the specific pathogen. It could come from a positive sample available in the laboratory or from a negative sample spiked with a solution of the specific pathogen. 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. Analysis protocol

⚠ Mix the buffers before use.

1. Extraction from swab

Samples	Mycoplasms / Influenza Virus / Infectious Bronchitis Virus
Preparation	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 by vortexing 10 sec / tube Transfer **50 µL** of the supernatant in a microtube or in a PCR microplate well
Lysis	Add **50 µL of LF3 buffer** and **2 µL of L2 buffer*** Cover and homogenize Incubate **5 minutes at +65 °C** then **15 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 step for the concerned kits. Refer to the corresponding package insert for preparation, storage, and use of the control.*

*Prior to use, a pre-mix of both reagents can be prepared and dispensed into each sample.
EPC-Ext is provided in the kit for the pathogen of interest.
Refer to the corresponding package insert for the preparation, storage, and use of the control.

2. Extraction from FTA CARD

Samples	Mycoplasms / Influenza Virus / Infectious Bronchitis Virus
Lysis	Cut a 3 mm² 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

3. Extraction from faces and environmental sample (dung scraping)

Sample	*Mycobacterium avium* **subsp.** ***paratuberculosis***
Preparation	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 3000 g** and remove the supernatant with a pipette. Add 300 mg of grinding 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**. Collect **50 µL** of supernatant.
Lysis	Add **50 µL of LF3 buffer, 2 µL of L2 buffer and 5 µL of EPC-Ext*** Cover and homogenize Incubate **5 minutes at +65 °C** then **15 minutes at +95 °C** Allow cooling to ensure the accuracy of subsequent pipetting

*Prior to use, a pre-mix of both reagents can be prepared and dispensed into each sample.
EPC-Ext is provided in the kit for the pathogen of interest.
Refer to the corresponding package insert for the preparation, storage, and use of the control.

4. 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.

V. Index of symbols

Symbol	Meaning
	Catalog 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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