ADIAPURE™ SLB

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

References:
ADIADP01S1-100 (100 ml)
ADIADP01S1-500 (500 ml)

English version
NE01S1-01
2020/01

ADIAPURE™ SLB

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
2020/01	NE01S1-01	N/A	First publication

I. General information

1. Purpose of the kit

ADIAPURE™ SLB is a nucleic acids DNA and RNA extraction kit based on the chemical lysis. The nucleic acids can be used without purification with the PCR amplification kit of ADIAVET range validated with the kit.

2. Description of test

Adiagene validated the ADIAPURE™ SLB kit from swabs for the detection of DNA pathogens in combination with the ADIAVET range.
The following table summarises the validated protocols.

		Swab
Avian diseases	*Mycoplasma gallisepticum*	X
	Mycoplasma synoviae	X
	Mycoplasma meleagridis	X
	Mycoplasma iowae	X
	Ornithobacterium rhinotracheale	X

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

II. Material & reagents

1. Composition of kit

The ADIAPURE SLB kit contains the following buffers:

REF ADIADP01S1-100		
L1	Lysis buffer	1 x 100 ml (ready-to-use)
L2	Enzyme	1 x 200 µl (ready-to-use)
L3	Lysis buffer	1 x 5 ml (ready-to-use)
REF ADIADP01S1-500		
L1	Lysis buffer	5 x 100 ml (ready-to-use)
L2	Enzyme	1 x 1000 µl (ready-to-use)
L3	Lysis buffer	1 x 25 ml (ready-to-use)

2. Validity and storage

On receipt, the L2 and L3 buffers should be aliquoted and stored at +2/8°C or at -<15°C.
The L1 buffer should be placed at room temperature in the darkness.
The L1 buffer can contains aggregate, warm it to obtain a clear solution before use.
Do not mix reagents of two different batches.
Do not freeze the L1 buffer.

⚠ **Mix the L3 buffer before use.**

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

III. Use of the samples and the controls

1. Precautions

Caution:
Prepare the buffers of 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 DNAs are quite sensitive molecules. Crude extracts should be stored at the end of extraction on melting ice or at +2/8°C for max. 24 hours, then at <-15°C.

3. Controls preparation

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

- The endogenous or exogenous internal control included in the ADIAVET™ kits allows validating the extraction and amplification steps of each sample.
- The positive control included in the ADIAVET™ kits allows validating 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 to include 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 positif 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. Extraction protocol

From swab

⚠ Mix the buffers before use.

Samples	**Mycoplasmes** and ***O. rhinotracheale***
Preparation	Cut **1 to 6 swabs** in a 5 ml tube. Add **1 ml of L1 buffer** if 1 to 3 swabs analyse **Or add 2 ml of L1 buffer** if 4 to 6 swabs analyse Mix by vortexing 10 sec / tube Transfer **50 µl** of the surpernatant in a microtube or in a well of PCR microplate
Lysis	Add **50 µl of L3 buffer** (beforehand mixed) and **2 µl of L2 buffer*** *The L2 buffer is optional but highly recommended for processing "difficult" samples, such as cloacal swabs.* Cover and homogenize. Incube **5 minutes at +65°C** *(if L2 buffer used) then* **15 minutes at +95°C.** Let to cool, to ensure the accuracy of subsequent pipetting.

*Just before using, a pre-mix of the both reagents could be prepared then added to each sample.

The swabs can be kept in L1 buffer at room temperature for 48h, for a longer conservation store at <-15°C.
The DNA solution can be kept at +2/8°C - 48 hours, for a longer conservation store at <-15°C.

V. Amplification

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

VI. Index of symbols

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

S.A.R.L. ADIAGENE
9, rue Gabriel Calloët-Kerbrat
22440 Ploufragan - France

RCS 417 876 299
Tel. +33 (0)2 96 68 40 20
www.biox.com