ADIAPURE™ PARATB MILK

DNA extraction and purification system from milk

References
ADIADP04M1-100 (100 tests)

English version - July 2016
NE04M1-05

ADIAPURE™ PARATB MILK

Main change since previous version

- **All the user manual :** Administrative → Changing logos

I. General information

1. ADIAPURE™ PARATB MILK

ADIAPURE™ PARATB MILK kit provides a fast and easy method for purification of total DNA from milk and increases the PCR* reliability with ADIAVET™ kits.
This kit has been developed to extract *M. avium* subsp. *paratuberculosis* DNA from bovine milk.
Purification requires no phenol/chloroform extraction or alcohol precipitation. DNA is ready for direct addition to qualitative or realtime PCR or can be stored several months at -20°C. The obtained DNA is purified from proteins, nucleases and PCR* inhibitors.

2. Description and principle of the test

First *M. avium* subsp. *paratuberculosis* are captured on magnetic beads, then bacteria are lyzed by grinding to destroy the cellular membrane, very resistant by mycobacteria.
Sensitivity threshold of ADIAPURE™ PARATB MILK extraction on bovine milk is between 6 and 60 bacteria/ml.

* The PCR process is covered by patents U.S. 4,683,195 and 4,683,202 (and their corresponding patents outside the US) owned by Hoffmann-La Roche, Inc.

II. Material and reagents

1. Reagents provided with the ADIAPURE™ PARATB MILK kit

Designations	Reagents	Storage	ADIADP04M1-100
L0	Lysis buffer L0	+2/8°C	1 x 110 ml bottle
L1	Lysis buffer L1	+2/25°C	1 x 65 ml bottle
L2	Lysis buffer L2	+2/8°C	2 x 1.1 ml tubes
Mb	Magnet beads	+2/8°C	1 x 2.5 ml bottle

2. Validity and storage

On receipt of this kit, L0, L2 reagents and Magnet beads Mb must be stored at +2/8°C. The reagent L1 should be stored at room temperature. All these reagents remain stable for at least 1 year. L1 reagent can precipitate. In this case, warm it until complete dissolving before use. Resuspend the magnetic beads by vortexing before use.

Do not mix reagents of different batches.
Do not freeze.

3. Equipment required but not supplied

- 15 ml tubes
- 1,5 ml microtubes
- 100-1000 µl, 1-20 µl, 20-200 µl pipettes
- Filter tips
- Sample mixer (mixing wheel)
-15 ml tubes magnet
- Latex gloves
- Vortex
- A universal laboratory grinder:

  Mixer Mill from Werder with Glass beads (Bio-X Diagnostics, 192 extractions: ref. ADIADPBIA-192 or 480 extractions: ref. ADIADPBIA-480)

  Or

  Fast Prep from MP Biomedical with Lysing Matrix B tubes (MP Biomedical, 100 extractions: ref. 116911.100)
- A centrifuge for microtubes
- 2 block heaters, water baths or incubators
- 10 ml and 2 ml sterile pipettes

4. Precautions - safety instructions

When working with chemicals, always wear a suitable lab coat and disposable gloves.

"Lysis buffer L0" contains Tris (tris hydroxy amino methane) (1-10%)
Signal word: WARNING

H315, H319, H335
P280, P305+P351+P338
Hazard statement(s):
H315 causes skin irritation
H319 causes serious eye irritation
H335 may causes respiratory irritation
Precautionary statement(s) :
P280: Wear protective gloves/protective clothing/eye protection/face protection
P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313: If eye irritation persists: Get medical advice/attention.
P302 + P352 : IF ON SKIN: Wash with plenty of water.

"Lysis buffer L2" contains a reagent at a concentration considered as dangerous: Proteinase K , 1,00%
Signal word: DANGER

H334
P261 / P280 / P342 + P311
Hazard statement(s):
H334 : May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Precautionary statement(s):
P261 : Avoid breathing dust/fume/gas/mist/vapours/spray.
P280 : Wear protective gloves/protective clothing/eye protection/face protection.
P342+P311 : If experiencing respiratory symptoms: Call a POISON CENTER/doctor.

We strongly recommend that only appropriately trained personnel perform this test. Ensure the accuracy and precision of the micropipettes used. The quality of the obtained results depends upon rigorous respect of good laboratory practices.

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.**

It is recommended to sue milk as fresh as possible. Freezing and defrosting decrease the sensitivity threshold.

III. Protocol

1. Mycobacteria capture

a- Place **10 ml** of raw milk as fresh as possible in a 15 ml tube and add **1 ml** of L0 buffer. Mix by vortexing.

b- Add **25 µl** of magnetic beads Mb suspension and gently mix for capture, for example on a rotating wheel at 10 rpm, during 30 minutes.

c- Magnetize during 10 minutes to catch all beads on one side of the tube. Carefully discard the liquid phase with a pipette.

d- Resuspend the beads pellet in **600 µl** of Lysis buffer L1.

2. Grinding with the Mixer Mill (Werder)

a- Put the whole mix into a microtube containing **300 mg** of glass beads.

b- Disrupt at 30 Hz for 10 minutes using a mixer mill. Centrifuge 5 minutes at 7500 g.

c- Place **300 µl** of the supernatant into a new 1.5 ml microtube and add **20 µl** of L2 buffer.

d- Incubate 10 minutes at 70°C then 15 minutes at +95°C.

The obtained DNA extract can be stored 24hours at +2/8°C or several months at -20°C+/-5°C.

3. Grinding with the Fast Prep (MP Biomedical)

a- Put the whole mix into a Lysing Matrix B tube containing glass beads.

b- Disrupt at 4m/sec for 3 x 45 seconds. Centrifuge 5 minutes at 7500 g.

c- Place **300 µl** of the supernatant into a new 1.5 ml microtube and add **20 µl** of L2 buffer.

d- Incubate 10 minutes at +70°C then 15 minutes at +95°C.

The obtained DNA extract can be stored 24 hours at +2/8°C or several months at -20°C+/-5°C.

You can now achieve the PCR process using the ADIAVET™ PARATB REAL TIME PCR kits.

IV. 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
	For veterinary in vitro use only – For animal use only

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