

Adia^X Control

Extraction Positive Control PARATB

Reference: ADC04EPC

Mycobacterium avium subsp. *paratuberculosis* positive control for extraction control

For veterinary *in vitro* use only



Kit composition

Content		ADC04EPC Kit 100 reactions
EPC PARATB	Extraction Positive Control <i>Mycobacterium avium</i> subsp <i>paratuberculosis</i>	1 lyophilized vial (To reconstitute)
NF-Water	Nuclease free water	1 x 500 µL tube (Ready to use)

Associated PCR kit(s)

Associated kit(s)	Reference(s)
ADIAVET™ PARATB REAL TIME	ADI045-100

Revision history

Date	Version	Modifications
02/2023	V01	First version

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

A. Test principle

The extraction positive control is made with an inactivated, lyophilized culture of *Mycobacterium avium* subsp. *paratuberculosis*.

This extraction positive control after rehydration can be used for two purposes:

- the extraction positive control can be used within each extraction series and allows the day-to-day extraction step control.
- it can be used to confirm the LOD_{METHOD}. It is calibrated between 1 and 100 the LOD_{METHOD}.

It is the responsibility of the laboratory to obtain the matrix free of *Mycobacterium avium* subsp. *paratuberculosis*.

B. Storage conditions

- On receipt, the kit should be stored dry at +2/8 °C.
- Reconstituted reagents should be stored at <-15 °C until the expiration date.
- Store away from sunlight.
- Do not mix reagents of two different batches.

C. Material required but not provided

- Real-time Thermal cycler and device.
- Instrument for homogenous mixing of tubes.
- Pipettes of 1 - 10 µL, 20 - 200 µL and 200 - 1000 µL.
- Nuclease-free filtered pipette tips.
- Nuclease-free microtubes of 1,5 mL and 2 mL.
- Powdered-free latex or nitrile gloves.
- Nuclease-free water.
- Associated PCR kits.
- Kit for nucleic acids extraction.
- Faeces free of *Mycobacterium avium* subsp. *paratuberculosis*.

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. Procedure

1. Reconstitution of the control

- Add **500 µL** of « **NF Water** » in « **EPC PARATB** » vial.
- Vortex vigorously at least 20 seconds.
- After reconstitution, the control is calibrated between **1 et 100 X LOD_{METHOD}**.
- Aliquot and store at <-15°C. Do not defrost more than 3 times.

2. LOD_{METHOD} confirmation

a. Preparation

Dilute extemporaneously to 1/10 the « **EPC PARATB** » with the NF Water = "EPC PARATB 1/10th". *Dilution should not be stored.*

b. Extraction

Minimum number session	Minimum number operator	Minimum number of repetitions of "EPC PARATB 1/10 th "
2	1	2

Spiking of matrix free of *Mycobacterium avium* subsp. *Paratuberculosis* according to the used protocol:

- Protocol without pre-filtration system:**

Spike 1 mL of Faeces suspension before the grinding step with 5 µL of "EPC PARATB 1/10th" solution.

- Protocol with Adiafilter system:**

Spike 10 mL of Faeces suspension before the pre-filtration step with 5 µL of "EPC PARATB 1/10th" solution.

- Protocol with Adiaprep system:**

Spike 1 mL of Faeces suspension before the centrifugation step with 5 µL of "EPC PARATB 1/10th" solution.

It is recommended to perform at least one negative extraction control per session to verify the absence of contamination.

c. Amplification

Nucleic acids are extracted with the associated Bio-X Diagnostics PCR kits according to kit instructions.

d. Validation of results

The four results obtained with « **EPC PARATB 1/10** » must be positive.

3. Extraction step control

a. Extraction:

Spiking of matrix free of *Mycobacterium avium* subsp. *Paratuberculosis* according to the used protocol:

- Protocol without pre-filtration system:**

Spike 1 mL of faeces suspension before the grinding step with 5 µL of "EPC PARATB" solution.

- Protocol with Adiafilter system:**

Spike 10 mL of faeces suspension before the pre filtration step with 5 µL of "EPC PARATB" solution.

- Protocol with Adiaprep system:**

Spike 1 mL of faeces suspension before the centrifugation step with 5 µL of "EPC PARATB" solution.

b. Amplification

Nucleic acids are extracted with the associated Bio-X Diagnostics PCR kits according to kit instructions.

c. Validation of results

All the obtained results during the different series of extraction constitute the control chart.

Symbols

Symbole	Signification
	Catalog number
	Manufacturer
	Temperature limitation
	Use by
	Batch code
	Consult Instructions for Use
	Contain sufficient for "n" tests
	For veterinary <i>in vitro</i> use only – For animal use only
	Keep away from sunlight