Automated Extraction of DNA from *Mycobacterium avium subspecies paratuberculosis* (MAP) in Fecal Samples with the KingFisher™ Flex (Low-Input Workflow)

Materials provided

Contents	Cat. No. TC-9119-480 (480 reactions)	Storage
VetAlert™ MagBead Proteinase K	25 mL	15-30°C (room temperature)
VetAlert™ MagBead Lysis Solution	190 mL	
VetAlert™ MagBead Wash Buffer	485 mL	
VetAlert™ MagBead Elution Buffer	45 mL	
VetAlert™ MagBead Resin	22 mL	
VetAlert™ MagBead Stabilization Buffer	100 mL	
VetAlert™ MagBead Stabilization Additive	2 mL	2-8°C (refrigerated)

Materials required but not provided

Reagents

- 100% ethanol, ACS reagent grade or equivalent
- 100% isopropanol, ACS reagent grade or equivalent
- Molecular biology grade water
- VetAlert™ MagBead Sample Disruption System (Tetracore, Inc. Cat.# TC-9131-100, includes Disruption Tubes and additional VetAlert™ MagBead Stabilization Buffer and Stabilization Additive)
- (Optional) VetAlert™ DNA Internal Control System [Tetracore, Inc. Cat.# TC-9123-100, includes VetAlert™ DNA Internal Control (DNA IC) and VetAlert™ Internal Control Primer Probe Mix]

Equipment and consumables

- Mini-BeadBeater 96 (BioSpec Products, Inc. Cat.#1001) or equivalent
- 2 mL microcentrifuge tubes
- KingFisher™ Flex Purification System (Thermo Fisher Cat.# 5400630)
- Micropipettes and sterile pipette tips with aerosol barriers
- Vortex mixer
- Microcentrifuge
- KingFisher™ 96 deep-well plates (Thermo Fisher Cat.# 95040460)
- KingFisher™ 96 well microplate, 200 µL (Thermo Fisher Cat.# 97002540)
- KingFisher ™ 96 tip comb for deep-well magnets (Thermo Fisher Cat.# 97002534)

Before you begin

Prepare Stabilization Solution:

- Prepare Stabilization Solution by combining 490 µL of Stabilization Buffer and 10 µL of Stabilization Additive per sample.

  Note: Prepare additional Stabilization Solution volume to accommodate pipetting loss.

Prepare 50% Ethanol:

- Dilute 100% ethanol to 50% with an equal volume of molecular biology grade water.

Preprocessing of samples

1. Add 0.3-0.4 g of fecal sample and 1 mL of molecular biology grade water to a 2 mL microcentrifuge tube.
2. Vortex vigorously for 3 minutes or until the sample is suspended.
3. Centrifuge at 100 x *g* for 30 seconds.
4. Add 500 µL of the prepared Stabilization Solution to a Disruption Tube.
5. Add 500 µL of the fecal supernatant to the Disruption Tube from step 4. The tube should be filled to within the grooves of the Disruption Tube. Overfilling can cause the tube to explode.
6. Briefly vortex the sample to allow the beads to mix with the supernatant.
7. Use the Mini-BeadBeater 96 (or equivalent) to bead beat the sample for 5 minutes at a speed setting 2400 rpm.
8. Centrifuge at 15,000 x *g* for 3 minutes.

Prepare purification plates and sample plate

9. **Wash Plate 1**: Add 100 µL of 50% ethanol and 900 µL of Wash Buffer to each well of a KingFisher™ 96 deep-well plate required for purification.
10. **Wash Plate 2**: Add 450 µL of 50% ethanol to each well of a KingFisher™ 96 deep-well plate required for purification.
11. **Elution Plate**: Add 50 µL of Elution Buffer to each well of a KingFisher™ 96 well microplate (200 µL) required for purification.
12. **Tip Comb Plate**: Place KingFisher™ 96 tip comb into an empty KingFisher™ 96 well microplate (200 µL).

13. **Sample Plate:**

    a. Add 30 µL of Proteinase K to each well of a KingFisher™ 96 deep-well plate required for purification.

    b. Transfer 300 µL of sample to a well containing Proteinase K.

    c. Add 300 µL of Lysis Solution to each sample well and pipette-mix.

       Optional: Add 6 µL of VetAlert™ DNA IC to the Lysis Solution if IC is used as an extraction control.

    d. Add 350 µL of 100% isopropanol to each well containing sample.

    e. Add 20 µL of Resin to each well containing sample.

       Note: The magnetic beads tend to drop out of suspension when not in use. Please ensure the Resin is completely resuspended prior to transfer by vigorously shaking or vortexing the bottle.

Begin process on the KingFisher Flex

14. Start KingFisher method ***Tetracore_VetAlert_MagBeadExtraction.bdz*** and follow instrument prompts for plate placement (see below).

Plate ID	Plate position on the KingFisher Flex
Sample Plate	1
Wash Plate 1	2
Wash Plate 2	3
Elution Plate	4
Tip Comb Plate	5

15. Follow prompts for removal of plates from the KingFisher Flex.

16. Transfer final eluates to a container of your choice for storage.

    Note: Extracted nucleic acid can be stored at 4°C (2°C to 8°C) for immediate use, or at -20°C (-15°C to -25°C) or -80°C (-60°C to -90°C) for long-term storage.

Symbols

Symbol	Meaning	Symbol	Meaning
	Catalog Number		Lot Number
	Consult Instructions for Use		Manufacturer
	Expiration Date		Temperature Limit

Revision History

Revision	Date	Description of Change
00	18 July 2024	Initial Release

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Revision: 18 July 2024
Part number: PLM-0801-1