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MAP Extraction System

For the extraction of DNA from bovine fecal samples or from culture media for downstream analysis by real-time PCR

NOTE: Three protocols are provided.

(Two bovine fecal protocols and one culture media protocol.)

For the bovine fecal protocols:

- **For maximum sensitivity use the Two Gram Protocol.**
- **If sensitivity is not as important as speed in processing samples, use the One Gram Protocol.**

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Product Description

MAP Extraction System
TC-9014-100

Materials Provided:

Component	Contents
Disruption Tubes	Microcentrifuge Tubes containing glass beads; 100 tubes/kit
Spin Columns	100 spin columns/kit
Collection Tubes	300 tubes/kit
NAB™ Buffer	1/kit
Binding Buffer	1/kit
Wash Buffer A	Supplied as concentrate. Add 100% Ethanol prior to use. 1/kit
Wash Buffer B	Supplied as concentrate. Add 100% Ethanol prior to use. 1/kit

Material and Equipment Required, but Not Provided:

- VetAlert™ Johne's Real-Time PCR (Tetracore Cat # TC-9828-100)
- DNase Free Water
- Centrifuges with rotors for 1.5mL to 2mL tubes and 50mL conical tubes
- 50mL sterile plastic conical tubes
- Beadbeating instruments such as Mini Beadbeater by BioSpec Product, Inc. or FastPrep® by Qbiogene, Inc.
- Micropipettes and sterile pipet tips with aerosol barriers
- DNase Free Microcentrifuge tubes and 50mL conical tubes
- Optional: 1xTE (10mM Tris-HCl, pH 8.0; 1mM EDTA)

Storage and Stability

The MAP Extraction System should be stored at ambient temperatures (20°C to 30°C) and is stable until the expiration date stated on the label.

Precautions

- Store and extract positive or suspected positive material separately from all PCR kit components, e.g. in separate rooms.
- Use sterile pipette tips with aerosol barriers to avoid potential sample-to-sample contamination.
- Periodically (at least weekly), disinfect all work area and pipettes with either a dilute bleach solution (i.e.,10% bleach) or ELIMINase® (Decon Labs). If using bleach, the 10% bleach solution should be made up fresh each week. Rinse surfaces with DNase free water after decontaminating surfaces.
- The Binding Buffer contains guanidinium thiocyanate and the Wash Buffer A contains guanidinium chloride. These are irritants and harmful. The Binding Buffer and Wash Buffer A react with bleach. Use care when handling the Binding Buffer and Wash Buffer A.

Limitations

This test should be used for extraction of DNA from bovine fecal samples or from culture media. No claims are made for other samples or for organisms derived from other animal species.

Specimen Collection and Storage

Fresh, moist fecal samples should be collected. Upon receipt in the testing laboratory the fecal samples should be processed immediately; refrigerated for up to 1 day and then processed; or aliquoted, frozen and stored at -80°C. Repeated freezing and thawing of fecal samples should be avoided, as DNA may degrade. Dry fecal samples can produce sub-optimal results and should be avoided.

Centrifugation Speeds

The centrifugation speeds listed in this protocol have been optimized for maximum nucleic acid recovery. The calculation for converting relative centrifugal force (RCF), which is measured in x g is:

$$RCF = 1.12 \times R \times (speed/1000)^2$$

RCF is the relative centrifugal force (in g), R is the radius of the centrifuge rotor in mm (for an Eppendorf 5415C model this is 73mm) and Speed is the RPM.

Preparation of Buffers

Occasionally, the Binding Buffer forms crystals. If crystals have formed, heat the Binding Buffer in a water bath or incubator to no hotter than 80°C for a few minutes. Then, vortex to resuspend the crystals.

Wash Buffer A and B are supplied as concentrates. Before using for the first time, add appropriate amount of 100% ethanol.

Component	Amount of 100% Ethanol
Wash Buffer A	50mL
Wash Buffer B	60mL

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PROTOCOLS

EXTRACTION OF BOVINE FECAL SAMPLES

Part I: Fecal Specimens

Two Gram Protocol: for Maximum Sensitivity

1. Transfer 2 grams (+/- 0.1g) of fecal material into a 50mL conical tube.
2. Add 35mL of DNase free water or 1 x TE to each fecal sample.
3. Vortex the sample for approximately 15 seconds (to break up large particles).
4. Rock the sample for 15 to 30 minutes at room temperature.
5. Incubate the sample at room temperature, without agitation, for 30 minutes (to allow larger particulate matter to settle to bottom of tube).
6. Remove the top 20mL and place into a new 50mL conical tube.
7. Centrifuge at 2500 x g for 10 minutes.
8. Using a serological pipet, carefully remove and discard the supernatant.
9. Resuspend the pellet with up to 1mL of 1 x TE.
10. Proceed to Part II: Lysis with either the Mini Beadbeater Protocol or the FastPrep Protocol on page 8.

One Gram Protocol: for Greater Processing Speed

1. Transfer 1 gram (+/- 0.1g) of fecal material into a 5mL Sarstedt tube.
2. Add 2mL of 1 x TE to each fecal sample.
3. Vortex the sample for approximately 15 seconds (to break up large particles).
4. Incubate the sample at room temperature, without agitation, for 5 minutes (to allow larger particulate matter to settle to bottom of tube).
5. Proceed to Part II: Lysis with either the Mini Beadbeater Protocol or the FastPrep Protocol on page 8.

Note: Pooling of Bovine Fecal Samples

Up to five fecal samples may be pooled together to reduce cost and effort when assessing herd status for Johne's disease. When coupled with VetAlert™ Johne's Real-Time PCR (Tetracore, TC-9828-100) pooling of up to five fecal samples will identify the presence of an infected animal(s) in the pool with minimal or no loss of sensitivity compared to individual animal testing.

To Pool Five Samples:

1. Process each sample according to Part I: Fecal Specimens (page 6), following steps 1-5.
2. At step 6, remove 4mL from each sample and place together into a new 50mL conical tube. (The total volume in the 50mL conical tube will be 20mL; 4mL from each of 5 samples.)
3. Continue the extraction procedure with the centrifugation in step 7 on page 6.

Part II: Lysis

Mini Beadbeater Protocol:

1. Place the fecal sample into a Disruption tube (containing glass beads). The tube should be filled to within the grooves of the disruption tube. Over filling can cause the tube to explode.
2. Briefly vortex the sample for a couple of seconds to allow the beads to mix with the supernatant.
3. Beadbeat the sample at 4800rpm for 5 minutes.
4. Continue to Part III: DNA Purification on page 9.

FastPrep Protocol:

1. Place the fecal sample into a Disruption tube (containing the glass beads). The tube should be filled to within the grooves of the disruption tube. Over filling can cause the tube to explode.
2. Briefly vortex the sample for a couple of seconds, to allow the beads to mix with the supernatant.
3. FastPrep the sample for 5 minutes at a speed setting of 5 m/s.
4. Continue to Part III: DNA Purification on page 9.

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Part III: DNA Purification

1. Centrifuge the sample in the Disruption tube for 10 minutes at 16,000 x g.
2. Remove the supernatant from the sample and place into a 2 mL microcentrifuge tube.
3. Add 100µL of NAB™ Buffer.
4. Invert the tube 5 times to mix.
5. Centrifuge at 1200 x g for 3 minutes.
   (This speed is crucial to the extraction of the nucleic acid. It may be necessary to optimize the centrifuge for optimal centrifugation speeds. To optimize the centrifuge, decrease and/or increase the g force by 100 x g steps until the optimal speed is determined).
6. Gently remove and discard the supernatant with pipet, being careful not to disturb the pellet. A large brown colored pellet will be visible in the bottom of the tube. A clear 'smear' of pellet may be visible directly above the large pellet. Do not disturb either the pellet or the 'smear.'
7. Add 560µl of Binding Buffer.
8. Vortex the sample for 5 seconds to loosely break up the pellet[1].
9. Incubate at room temperature for 10 minutes.
10. Add 560µl of 100% ethanol to the sample.
11. Mix the sample by vortexing for a few seconds[2].
12. Add 630µl of the sample to a spin column (placed in a clean 2mL collection tube).
13. Centrifuge the sample at 5,200 x g for 1 minute.
14. Repeat steps 12 and 13 with the remaining sample.
15. Add 500µl of Wash Buffer A to the spin column (place column into a clean collection tube).
16. Centrifuge the sample at 5,200 x g for 1 minute.

[1] The pellet may not completely break up; the 'smear' should dissolve.
[2] If the pellet is not disrupted, pipet the solution up and down to break up the pellet. The pellet may not completely resuspend; however, it should be broken into small pieces.

17. Add 500µl of Wash Buffer B to the spin column (place column into a clean collection tube).
18. Centrifuge the sample at 12,000 x g for 3 minutes.
19. Centrifuge the sample (without buffers) at 16,000 x g for 1 minute to remove any residual Wash Buffer B.
20. Place the spin column into 1.5 ml Eppendorf microcentrifuge tubes. Discard the old collection tubes.
21. Add 50µl of dH_2O to the spin column to elute the DNA.
22. Incubate the sample at room temperature for 1 minute.
23. Centrifuge the sample at 5,200 x g for 1 minute. Save the eluant. (Discard the spin column).
24. The sample is ready to PCR using the VetAlert™ Johne's Real-Time PCR.
25. Extracted DNA can be stored at 4°C for up to 48 hours or at -20°C for extended storage.

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EXTRACTION OF LIQUID CULTURE SAMPLES

Culture Confirmation Protocol

Mini BeadBeater Protocol:

1. Place 1mL (+/- 100µL) of broth culture into the Disruption tube (containing the glass beads).
2. Briefly vortex the sample for a couple of seconds, to allow the beads to mix with the supernatant.
3. Beadbeat the sample at 4800rpm for 5 minutes.
4. Centrifuge the sample for 10 minutes at 16,000 x g.
5. Remove the supernatant and transfer to a new microcentrifuge tube. (Discard the Disruption tube.)
6. The sample is ready to PCR using the VetAlert™ Johne's Real-Time PCR.
7. Extracted DNA can be stored at 4°C for up to 48 hours or at -20°C for extended storage.

FastPrep Protocol:

1. Place 1mL (+/- 100µL) of broth culture into the Disruption tube (containing the glass beads).
2. Briefly vortex the sample for a couple of seconds, to allow the beads to mix with the supernatant.
3. FastPrep the sample at 5minutes at a speed setting of 5 m/s.
4. Centrifuge the sample for 10 minutes at 16,000 x g.
5. Remove the supernatant and transfer to a new microcentrifuge tube. Discard the Disruption tube.
6. The sample is ready to PCR using the VetAlert™ Johne's Real-Time PCR.
7. Extracted DNA can be stored at 4°C for up to 48 hours or at -20°C for extended storage.

* For most culture media this extraction is sufficient. However if the culture media contains high levels of egg yolk (which may inhibit the PCR reaction) it may be necessary to further purify the DNA. In that case, 200µL of the material should be processed according to the DNA Purification Part III on page 8, beginning at step #7. (Starting with the addition of 560µL of Binding Buffer to 200µL of sample.)

Manufactured by

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