MagMAX™ Pathogen RNA/DNA Kit

For fecal samples

Catalog Numbers 4462359

Pub. No. 4466370 **Rev.** B

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *MagMAX™ Pathogen RNA/DNA Kit User Guide* (Pub. No. 4463379). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This document provides procedures for purifying nucleic acid on the KingFisher™ Flex Magnetic Particle Processor, MagMAX™ Express-96 Deep Well Magnetic Particle Processor (MME-96 processor), or the MagMAX™ Express Magnetic Particle Processor (MME-24 processor). For manual purification procedures, see the *MagMAX™ Pathogen RNA/DNA Kit User Guide* (Pub. No. 4463379).

You can use the MagMAX™ Pathogen RNA/DNA Kit to purify nucleic acid from 115 µL or 400 µL of clarified fecal lysate. We have evaluated this protocol on fecal samples from a limited number of animals; your results can vary.

Before each use of the kit

When preparing the reagents:

- Calculate the total volume that is required for each component: volume for 1 reaction × the total number of reactions.
- Include 10% excess volume to account for pipetting errors.

Prepare Lysis/Binding Solution

1. Combine the components listed below in the order indicated.

Component	Volume
Lysis/Binding Solution Concentrate	500 µL
Carrier RNA (µg/µL)	2 µL
(Optional)[1] Xeno™ RNA or Xeno™ DNA	2 µL
Total volume for 1 reaction	**504 µL**

[1] Add nuclease-free water if not adding Xeno™ RNA or DNA.

2. Mix well by vortexing.

Prepare the Bead Mix

1. Vortex the Nucleic Acid Binding Beads well to ensure that the beads are fully resuspended.
2. On ice, combine the components that are listed below.

Component	Volume
Nucleic Acid Binding Beads	10 µL
Lysis ENHANCER	10 µL
Total volume for 1 reaction	**20 µL**

3. Mix well by vortexing.

   Store on ice for up to 4 hours.

Prepare a fecal suspension

1. Add 0.4 g to 0.5 g of fecal sample to a 2-mL microcentrifuge tube.
2. Add 1 mL of 1X PBS to the tube.
3. Vortex vigorously (maximum setting) for 3 minutes, until the solution is fully suspended.
4. Centrifuge at 100 *x g* (low setting) for ~30 seconds to collect the solution at the tube bottom.

Proceed to:

- "Prepare the lysate using microcentrifuge tubes" on page 2 — Recommended for up to 24 samples
- "Prepare the lysate using plates" on page 2 — Recommended for more than 24 samples

Prepare the lysate using microcentrifuge tubes

This method is recommended for up to 24 samples.

Note: A wide bore tip can help making pipetting easier is pipette tips get clogged from fecal material.

For each sample:

1. Add 500 µL of the prepared Lysis/Binding Solution to a 1.5-mL microcentrifuge tube.
2. Add 200 µL of the fecal suspension to the tube.
3. Vortex vigorously (maximum setting) for 5 minutes.
4. Centrifuge at 16,000 × *g* (maximum setting) for 3 minutes to clarify the lysate.

   After 3 minutes, particulates should not be visible.
5. *(Optional)* If particulates are visible, centrifuge at 16,000 *x g* for 2 minutes.

Proceed to "Process samples on the KingFisher™ Flex/MagMAX™ Express-96 Deep Well Magnetic Particle Processor" on page 3 or "Process samples on the MagMAX™ Express Magnetic Particle Processor (MME-24)" on page 4.

Prepare the lysate using plates

This method is recommended for more than 24 samples.

Note: A wide bore tip can help making pipetting easier is pipette tips get clogged from fecal material.

1. Add 500 µL of the prepared Lysis/Binding Solution to each reaction well of a MagMAX™ Express-96 Deep Well Plate.
2. Add 200 µL of the fecal suspension to each reaction well.
3. Cover the plate with an Aluminum Adhesive Plate Sealer.
4. Using a plate shaker, shake at vigorous speed for 5 minutes (see the *MagMAX™ Pathogen RNA/DNA Kit User Guide*, Part. No. 4463379, for shaker settings).
5. Centrifuge at ≥2500 *x g* (maximum setting) for 5 minutes to clarify the lysate.

Proceed to "Process samples on the KingFisher™ Flex/MagMAX™ Express-96 Deep Well Magnetic Particle Processor" on page 3 or "Process samples on the MagMAX™ Express Magnetic Particle Processor (MME-24)" on page 4.

Process samples on the KingFisher™ Flex/MagMAX™ Express-96 Deep Well Magnetic Particle Processor

Note: It is critical that you prepare the sample plate last to reduce the time that the Bead Mix, sample, and 100% isopropanol are unmixed. To ensure best results, after preparing the sample plate, immediately load it onto the processor for purification.

Complete the steps below at room temperature and in the order indicated.

1. Select one of the following scripts.

Volume	Script	Instrument
115-µL clarified lysate	4462359_DW_50	• KingFisher™ Flex Magnetic Particle Processor • MagMAX™ Express-96 Deep Well Magnetic Particle Processor
	MagMAX™_Pathogen_Stnd_Vol	• KingFisher™ Flex Magnetic Particle Processor
400-µL clarified lysate	4462359_DW_HV	• KingFisher™ Flex Magnetic Particle Processor • MagMAX™ Express-96 Deep Well Magnetic Particle Processor
	MagMAX™_Pathogen_High_Vol	• KingFisher™ Flex Magnetic Particle Processor

2. Prepare the tip comb plate: Place an MME-96 Deep Well Tip Comb in one MME-96 Standard Plate.

3. Prepare the Wash Solution 1 plates:
   - For 115 µL of clarified lysate, add 150 µL of prepared Wash Solution 1 to two MME-96 Standard Plates.
   - For 400 µL of clarified lysate, add 300 µL of prepared Wash Solution 1 to two MME-96 Deep Well Plates.

4. Prepare the Wash Solution 2 plates:
   - For 115 µL of clarified lysate, add 150 µL of prepared Wash Solution 2 to two MME-96 Standard Plates.
   - For 400 µL of clarified lysate, add 450 µL of prepared Wash Solution 2 to two MME-96 Deep Well Plates.

5. Prepare the elution plate by adding 90 µL of Elution Buffer to one MME-96 Standard Plate (not the tip comb plate).

6. Prepare the sample plate:

   Note: After you start preparing the sample plate, do not pause until all plates are loaded onto the MME-96 processor and you have started the script.

   a. Add 20 µL of prepared Bead Mix to one MME-96 Deep Well Plate.

   b. Add prepared sample (clarified lysate) to the plate according to the plate processing setup table below.

c. Add 100% isopropanol to the plate according to the plate processing setup table below.

7. Immediately start the MME-96 processor script, then load the plates onto the processor as directed.

Table 2 Plate processing setup: Fecal 115 µL (deep-well head configuration)

Plate ID	Plate position	Plate type	Reagent	Volume per well
Sample plate	1	MME-96 Deep Well Plate	Bead Mix	20 µL
			Sample (clarified lysate)	115 µL
			100% Isopropanol	65 µL
First Wash 1	2	MME-96 Standard Plate	Wash Solution 1	150 µL
Second Wash 1	3	MME-96 Standard Plate	Wash Solution 1	150 µL
First Wash 2	4	MME-96 Standard Plate	Wash Solution 2	150 µL
Second Wash 2	5	MME-96 Standard Plate	Wash Solution 2	150 µL
Elution	6	MME-96 Standard Plate	Elution Buffer	90 µL
Tip comb plate	7	MME-96 Standard Plate	MME-96 Deep Well Tip Comb in standard plate	

Table 3 Plate processing setup: Fecal 400 µL (deep-well head configuration)

Plate ID	Plate position	Plate type	Reagent	Volume per well
Sample plate	1	MME-96 Deep Well Plate	Bead Mix	20 µL
			Sample (clarified lysate)	400 µL
			100% Isopropanol	350 µL
First Wash 1	2	MME-96 Deep Well Plate	Wash Solution 1	300 µL
Second Wash 1	3	MME-96 Deep Well Plate	Wash Solution 1	300 µL
First Wash 2	4	MME-96 Deep Well Plate	Wash Solution 2	450 µL
Second Wash 2	5	MME-96 Deep Well Plate	Wash Solution 2	450 µL
Elution	6	MME-96 Standard Plate	Elution Buffer	90 µL
Tip comb plate	7	MME-96 Standard Plate	MME-96 Deep Well Tip Comb in standard plate	

STOPPING POINT Store the purified nucleic acid on ice for immediate use, at –20°C for up to 1 month, or at –80°C for long-term storage.

Process samples on the MagMAX™ Express Magnetic Particle Processor (MME-24)

Complete the steps below at room temperature and in the order indicated.

1. Select the **4462359** MME-24 script.
2. Insert the MME-24 Tip Combs into the instrument head.
3. Add 150 µL of prepared Wash Solution 1 to rows B and C of an MME-24 Plate.
4. Add 150 µL of prepared Wash Solution 2 to rows D and E.
5. Add 90 µL of Elution Buffer to row F.
6. To row A, add the following in the order indicated:
   a. 20 µL of prepared Bead Mix.
   b. 115 µL of prepared sample (clarified lysate).

c. 65 µL of 100% isopropanol.

7. Load the MME-24 Plate onto the processor, then start the MME-24 processor script.

Table 4 Processing plate setup: Feces

Row	Reagent	Volume per well
A	Bead Mix	20 µL
	Sample (clarified lysate)	115 µL
	100% Isopropanol	65 µL
B	Wash Solution 1	150 µL
C	Wash Solution 1	150 µL
D	Wash Solution 2	150 µL
E	Wash Solution 2	150 µL
F	Elution Buffer	90 µL

STOPPING POINT Store the purified nucleic acid on ice for immediate use, at –20°C for up to 1 month, or at –80°C for long-term storage.

Limited product warranty

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Revision history: Revision history of Pub. no. 4466370

Revision	Date	Description
B	12 January 2018	• Added instructions for KingFisher™ Instruments. • Updated to the current document template, with associated updates to the warranty, trademarks, and logos.
A	June 2011	New document