MagMAX™ Pathogen RNA/DNA Kit

For InPouch™ TF culture samples

Catalog Numbers 4462359

Pub. No. 4466371 **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 Biomed Diagnostics InPouch™ TF *(Tritrichomonas foetus)* culture. You can use:

- 300 µL of InPouch™ TF culture
- 115 µL of clarified InPouch™ TF culture lysate

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	InPouch™TF Culture	Clarified Lysate
	Volume	Volume
Lysis/Binding Solution Concentrate	350 µL	150 µL
Carrier RNA (µg/µL)	2 µL	1 µL
(Optional)[1] Xeno™ RNA or Xeno™ DNA	2 µL	2 µL
100% Isopropanol	350 µL	—
Total volume for 1 reaction	**704 µL**	**153 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.

Sample preparation method — InPouch™ TF culture

Use this sample preparation method if you will be purifying nucleic acid directly from the InPouch™ TF culture. If you will be purifying nucleic acid from clarified lysate, see "Sample preparation method — clarified lysate" on page 2.

For each sample:

1. Mix the InPouch™ TF culture well by gently pulling the pouch up and down across the edge of a table 6 to 8 times.
2. Using sterilized scissors, cut the top of the pouch lower chamber.

   Note: Be sure to wipe the scissor blades with 10% bleach in between pouches.
3. Aspirate 300 µL from the pouch, then proceed immediately to the purification procedure for the MagMAX™ Express-96 Deep Well Magnetic Particle Processor and follow the MME-96 script for 300 µL of InPouch™ TF culture on "Process samples on the KingFisher™ Flex/MagMAX™ Express-96 Deep Well Magnetic Particle Processor: InPouch™ TF culture" on page 4.

Sample preparation method — clarified lysate

Use this sample preparation method if you will be purifying nucleic acid from the clarified lysate. If you will be purifying nucleic acid directly from the InPouch™ TF culture, see "Sample preparation method — InPouch™ TF culture" on page 2.

Prepare a suspension

For each sample:

1. Mix the InPouch™ TF culture well by gently pulling the pouch up and down across the edge of a table 6 to 8 times.
2. Using sterilized scissors, cut the top of the pouch lower chamber.

   Note: Be sure to wipe the scissor blades with 10% bleach in between pouches.
3. Using a sterile, disposable pipette, transfer the entire sample into a 5-mL conical tube.
4. Cap the tube, place into a tube adaptor, then centrifuge at ≥2250 *x g* (maximum setting) for 3 minutes.
5. Using a disposable pipette, carefully aspirate, then discard all supernatant without disturbing the pellet.
6. Add 1000 µL of sterile 1X PBS to the tube.

Proceed to:

- "Prepare the lysate using microcentrifuge tubes" on page 3 — Recommended for up to 24 samples
- "Prepare the lysate using plates" on page 3 — Recommended for more than 24 samples

Prepare the lysate using microcentrifuge tubes

This method is recommended for up to 24 samples.

For each sample:

1. Add 150 µL of the prepared Lysis/Binding Solution to a 1.5-mL microcentrifuge tube.
2. Vortex the pellet suspension vigorously (maximum setting) for 15 seconds.
3. Add 100 µL of the vortexed suspension to the 1.5-mL microcentrifuge tube containing Lysis/Binding Solution.
4. Vortex vigorously (maximum setting) for 3 minutes.
5. Centrifuge at 16,000 *x g* (maximum setting) for 2 minutes to clarify the lysate.

Note: If you are using the MagMAX™ Express-96 Deep Well Magnetic Particle Processor, use the MME-96 script for 115 µL of clarified lysate on (see "Process samples on the KingFisher™ Flex/MagMAX™ Express-96 Deep Well Magnetic Particle Processor: Clarified lysate" on page 5).

Proceed to one of the following:

- "Process samples on the KingFisher™ Flex/MagMAX™ Express-96 Deep Well Magnetic Particle Processor: InPouch™ TF culture" on page 4
- "Process samples on the KingFisher™ Flex/MagMAX™ Express-96 Deep Well Magnetic Particle Processor: Clarified lysate" on page 5
- "Process samples on the MagMAX™ Express Magnetic Particle Processor (MME-24): clarified lysate" on page 5

Prepare the lysate using plates

This method is recommended for more than 24 samples.

1. Add 150 µL of the prepared Lysis/Binding Solution to each reaction well of a MagMAX™ Express-96 Deep Well Plate.
2. Vortex the pellet suspension vigorously (maximum setting) for 15 seconds.
3. Add 100 µL of the vortexed suspension to each reaction well.
4. Cover the plate with an Aluminum Adhesive Plate Sealer.
5. 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).
6. Centrifuge at ≥2500 *x g* (maximum setting) for 5 minutes to clarify the lysate.

Note: If you are using the MagMAX™ Express-96 Deep Well Magnetic Particle Processor, use the MME-96 script for 115 µL of clarified lysate on (see "Process samples on the KingFisher™ Flex/MagMAX™ Express-96 Deep Well Magnetic Particle Processor: Clarified lysate" on page 5).

Proceed to one of the following:

- "Process samples on the KingFisher™ Flex/MagMAX™ Express-96 Deep Well Magnetic Particle Processor: InPouch™ TF culture" on page 4
- "Process samples on the KingFisher™ Flex/MagMAX™ Express-96 Deep Well Magnetic Particle Processor: Clarified lysate" on page 5
- "Process samples on the MagMAX™ Express Magnetic Particle Processor (MME-24): clarified lysate" on page 5

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

Note: It is critical that you prepare the sample plate last to reduce the time that the Bead Mix, sample, and Lysis/Binding Solution 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 the one of the following scripts.

Script	Instrument
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, by adding 300 µL of prepared Wash Solution 1 to two MME-96 Deep Well Plates.
4. Prepare the Wash Solution 2 plates, by adding 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 300 µL of prepared sample to the plate.
   c. Add 700 µL of prepared Lysis/Binding Solution to the plate.
7. Immediately start the MME-96 processor script, then load the plates onto the processor as directed.

Table 1 Processing plate setup: InPouch™ TF culture (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	300 µL
			Lysis/Binding Solution	700 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 1	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 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 KingFisher™ Flex/MagMAX™ Express-96 Deep Well Magnetic Particle Processor: Clarified lysate

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 the one of the following scripts.

Script	Instrument
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

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, by adding 150 µL of prepared Wash Solution 1 to two MME-96 Standard Plates.
4. Prepare the Wash Solution 2 plates, by adding 150 µL of prepared Wash Solution 2 to two MME-96 Standard 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 115 µL of prepared sample (clarified lysate) to the plate.
   c. Add 65 µL 100% isopropanol to the plate.
7. Immediately start the MME-96 processor script, then load the plates onto the processor as directed.

Table 2 Processing plate setup: clarified lysate (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 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): clarified lysate

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 3 Processing plate setup: InPouch™ TF culture- clarified lysate

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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Manufacturer: Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania

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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