MagMAX™ Pathogen RNA/DNA Kit

For oral fluid samples

Catalog Numbers 4462359

Pub. No. 4466369 **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 or MagMAX™ Express-96 Deep Well Magnetic Particle Processor (MME-96 processor). For manual purification procedures, see the *MagMAX™ Pathogen RNA/DNA Kit User Guide* (Pub. No. 4463379).

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	450 µL
Carrier RNA (µg/µL)	2 µL
(Optional)[1] Xeno™ RNA or Xeno™ DNA	2 µL
Total volume for 1 reaction	**454 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 the lysate using microcentrifuge tubes

This method is recommended for up to 24 samples.

For each sample:

1. Add 450 µL of the prepared Lysis/Binding Solution to a 1.5-mL microcentrifuge tube.
2. Vortex the oral fluid vigorously (maximum setting) for 15 seconds.
3. Add 300 µL of the vortexed oral fluid to the 1.5-mL microcentrifuge tube containing the 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.

Proceed to "Process samples on the KingFisher™ Flex/MagMAX™ Express-96 Deep Well Magnetic Particle Processor" on page 2.

Prepare the lysate using plates

This method is recommended for more than 24 samples.

1. Using a multichannel pipette, add 450 µL of the prepared Lysis/Binding Solution to each reaction well of a MagMAX™ Express-96 Deep Well Plate.
2. Vortex the oral fluid vigorously (maximum setting) for 15 seconds.
3. Add 300 µL of the vortexed oral fluid to each reaction well.
4. Cover the plate with an Aluminum Adhesive Plate Sealer.
5. Using a plate shaker, shake at moderate 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.

Proceed to "Process samples on the KingFisher™ Flex/MagMAX™ Express-96 Deep Well Magnetic Particle Processor" on page 2.

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

For oral fluid samples, we determined that 300 µL is the optimal input volume. Because MME-96 Deep Well Plates are required for the volume that is used with this workflow, we have not included MagMAX™ Express Magnetic Particle Processor (MME-24) or manual purification procedures.

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_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 600 µL of prepared sample (clarified lysate) to the plate.

   c. Add 350 µL of 100% isopropanol 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: Oral fluid (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)	600 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 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.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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

Revision history: Pub. No. 4466369

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