MagMAX™ Pathogen RNA/DNA Kit

For low-cell-content samples

Pub. No. 4463378 **Rev.** C

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), and 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 50, 100, 200, or 300 µL of the following low-cell-content samples:

- Serum

  Note: The serum should be relatively free of red blood cells. If the serum contains a significant number of red blood cells, we recommend that you follow the whole blood procedure (see the *MagMAX™ Pathogen RNA/DNA Kit User Guide*, Part. No. 4463379).
- Plasma
- Swabs (nasal, tracheal, and cloacal)
- Ear notches

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 the Lysis/Binding Solution

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

Component	Starting sample volume			
	50 µL	**100 µL**	**200 µL**	**300 µL**
Lysis/Binding Solution Concentrate	65 µL	125 µL	250 µL	350 µL
Carrier RNA (µg/µL)	1 µL	2 µL	2 µL	2 µL
(Optional)[1] Xeno™ RNA or Xeno™ DNA	2 µL	2 µL	2 µL	2 µL
100% Isopropanol	65 µL	125 µL	250 µL	350 µL
Total volume for 1 reaction	**133 µL**	**254 µL**	**504 µL**	**704 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 dry swabs

1. Add 750 µL of Viral Transport Media (VTM) to a 2-mL microcentrifuge tube.
2. Place the swab tip into the microcentrifuge tube, then cut away the swab shaft.
3. If needed, add more VTM to submerge the swab tip.
4. Close the tube, then vortex vigorously (maximum setting) for 1 minute.

IMPORTANT! If you are testing for bacteria, skip step 5 and 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. If centrifugation is performed, the bacterial samples will be lost, as the bacteria will be pelleted with the other waste.

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5. Centrifuge at 16,000 *x g* (maximum setting) for 30 seconds.

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 swabs in VTM

If the swabs have been transported in tubes that contain VTM, vortex the tubes vigorously (maximum setting) for 1 minute.

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 dry ear notches

1. Place each dry ear notch sample into separate 2-mL microcentrifuge tubes.
2. Add 1 mL of 1X PBS, pH 7.4, to each tube.
3. Place the tubes on a vortexer with a vortex adapter or Disrupter Genie, then vortex vigorously (maximum setting) for 10 minutes at room temperature.
4. Centrifuge at 10,000 *x g* (maximum setting) for 30 seconds to collect the tube contents.

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 ear notches in PBS

If the swabs have been transported in tubes that contain PBS, vortex the tubes vigorously (maximum setting) for 1 minute.

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 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 one of the following scripts.

Volume	Script
50-µL sample	4462359_DW_50
100-, 200-, or 300-µL samples	4462359_DW_HV

2. Prepare the tip comb plate: Place an MME-96 Deep Well Tip Comb in one Standard Plate (96–well).
3. Prepare the Wash Solution 1 plates:
   - For 50-µL sample volumes, add 150 µL of prepared Wash Solution 1 to two MME-96 Standard Plates.
   - For 100-, 200-, or 300-µL sample volumes, add 300 µL of prepared Wash Solution 1 to two Deep Well Plates (96–well).
4. Prepare the Wash Solution 2 plates:
   - For 50-µL sample volumes, add 150 µL of prepared Wash Solution 2 to two MME-96 Standard Plates.
   - For 100-, 200-, or 300-µL sample volumes, 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 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 to the plate according to Table 1 or Table 2.

c. Add prepared Lysis/Binding Solution to the plate according to Table 1 or Table 2.

7. Immediately start the processor script, then load the plates onto the processor as directed. Use the Magnetic Head for the 96 Deep-Well Plate.

Table 1 Processing plate setup: Low-cell-count samples with volume 50 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	50 µL
			Lysis/ Binding Solution	130 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	

Table 2 Processing plate setup: Low-cell-count samples with volume > 50 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	20 µL	20 µL
			Sample	100 µL	200 µL	300 µL
			Lysis/ Binding Solution	250 µL	500 µL	700 µL
First Wash 1	2	MME-96 Deep Well Plate	Wash Solution 1	300 µL	300 µL	300 µL
Second Wash 1	3	MME-96 Deep Well Plate	Wash Solution 1	300 µL	300 µL	300 µL
First Wash 2	4	MME-96 Deep Well Plate	Wash Solution 2	450 µL	450 µL	450 µL
Second Wash 2	5	MME-96 Deep Well Plate	Wash Solution 2	450 µL	450 µL	450 µL
Elution	6	MME-96 Standard Plate	Elution Buffer	90 µL	90 µL	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)

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. 50 µL of prepared sample.

c. 130 µL of prepared Lysis/Binding Solution.

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

Table 3 Processing plate setup: Low cell content

Row	Reagent	Volume per well
Row A	Bead Mix	20 µL
	Sample	50 µL
	Lysis/Binding Solution	130 µL
Row B	Wash Solution 1	150 µL
Row C	Wash Solution 1	150 µL
Row D	Wash Solution 2	150 µL
Row E	Wash Solution 2	150 µL
Row 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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The information in this guide is subject to change without notice.

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