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MagMAX™ Pathogen RNA/DNA Kit

USER GUIDE

for use with:
KingFisher™ Apex Purification System
KingFisher™ Duo Prime Purification System
KingFisher™ Flex Purification System

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For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0030202 D (English)

Revision	Date	Description
D	31 January 2024	• The MagMAX™ Express-96 Deep Well Magnetic Particle Processor was removed from this document due to product discontinuation. • The regulatory statement was updated.
C	12 January 2018	• Added instructions for KingFisher™ Instruments • Updated to the current document template, with associated updatesto the warranty, trademarks, and logos.
B	1 February 2011	New document for MagMAX™ Pathogen RNA/DNA KitMagMAX™ Pathogen RNA/DNA Kit.

The information in this guide is subject to change without notice.

Contents

1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Purpose of the product

The MagMAX™ Pathogen RNA/DNA Kit is designed for rapid purification of nucleic acid (RNA and DNA) from a wide variety of sample types.

Compatible methods

Method	Throughput	Compatible instrument
Automation	96 samples at one time	• KingFisher™ Flex Magnetic Particle Processor • KingFisher™ Apex Purification System
Automation	24 samples at one time	• KingFisher™ Flex Magnetic Particle Processor • KingFisher™ Apex Purification System
Manual	Up to 96 samples at one time	—-

Sample types and input volume

Sample type	Sample input volume		
	Flex processor or 96DW processor head	24DW processor head	Manual processing
Low-cell-content [1]	50, 100, 200, or 300 µL	50 µL	50 µL
Semen[2]	115 µL	115 µL	115 µL
Feces [2]	115 or 400 µL	115 µL	115 µL
InPouch™ TF culture [2]	115 or 300 µL	115 µL	115 µL

(continued)

Sample type	Sample input volume		
	Flex processor or 96DW processor head	24DW processor head	Manual processing
Whole blood	100 µL	—	—
Oral fluid [2]	600 µL	—	—

[1] Serum, plasma, swabs (nasal, tracheal, and cloacal),and ear notches.
[2] Clarified lysate volumes are listed.

Contents and storage

Upon receipt, store each component as indicated below.

IMPORTANT! Do not freeze the Nucleic Acid Binding Beads.

Contents	Amount	Storage
Lysis/Binding Solution Concentrate	240 mL	15—30°C
Wash Solution 1 Concentrate	250 mL [1]	
Wash Solution 2 Concentrate	2 x 58 mL [1]	
Elution Buffer	60 mL	
Nucleic Acid Binding Beads	5.5 mL	4°C [2]
Carrier RNA	5 x 220 µL	–20°C
Lysis ENHANCER	5.5 mL	

[1] Before use, consult “Before first use of the kit” on page 11.
[2] Do not freeze.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Reagents

Item	Source	Sample type					
		Low-cell-content	Whole blood	Semen	Oral fluid	Feces	InPouch™ TF culture
100% Ethanol, ACS reagent grade or equivalent	MLS	X	X	X	X	X	X
100% Isopropanol, ACS reagent grade or equivalent	MLS	X	X	X	X	X	X
Viral Transport Media (VTM)	MLS	X					
(Optional) Xeno™ RNA Control	A29763	X	X	X	X	X	X
(Optional) Xeno™ DNA Control	A29764	X	X	X	X	X	X
1X PBS, pH 7.4 (sterile)	MLS					X	X

Instruments, plates, and combs

Item	Source
High-throughput: up to 96 samples (96-well format)	
KingFisher™ Flex Purification System	5400630
KingFisher™ Apex Purification System	5400920
Deep Well Plates	95040450 95040450B
Standard Plates	97002540 97002540B
Deep Well Tip Combs	97002534 97002534B
Low-throughput: up to 24 samples	
KingFisher™ Flex Purification System	5400640
KingFisher™ Apex Purification System	5400940

(continued)

Item	Source
Plates (Plate)	95040470 and 95040480 (sterile option) 95040470B
Tip Combs (Tip Combs)	97002610 97002610B

General laboratory equipment

Item	Source	Sample type					
		Low-cell-content	Whole blood	Semen	Oral fluid	Feces	InPouch™ TF culture
Disposable gloves	MLS	X	X	X	X	X	X
Pipette tips[1]							
P1000	MLS	X	X	X	X	X	X
Large-bore	MLS		X	X		X	
Pipettes							
Single- and multichannel pipettes	MLS	X	X	X	X	X	X
(Optional) Disposable serological pipettes (5- to 50-mL), or equivalent, and a pipetting device for the serological pipettes	MLS	X	X	X	X	X	X
Repeater pipettes	MLS		X	X	X	X	X
Disposable pipettes, sterile	MLS				X		X
Microcentrifuge tubes and accessories							
1.5-mL	MLS	X	X	X	X	X	X
2-mL	MLS	X	X	X		X	
Conical-Bottom Centrifuge Tubes, Polypropylene, 5-mL	MLS						X
Microcentrifuge (capable of 16,000 × *g*)	MLS	X	X	X	X	X	X
Plate centrifuge, with tube adaptors	MLS		X	X	X	X	X

(continued)

Item	Source	Sample type					
		Low-cell-content	Whole blood	Semen	Oral fluid	Feces	InPouch™ TF culture
Aluminum Adhesive Plate Sealer	MLS		X	X	X	X	X
Barnstead/Lab-Line Titer Plate Shaker	MLS	X	X	X	X	X	X
Vortexer	MLS	X	X	X	X	X	X
Vortex adaptor	AM10024	X	X	X	X	X	X

[1] Pipette tips should be aerosol-resistant and nuclease-free.

2 Automation methods

For manual purification procedures, see Appendix B, “Manual methods”.

Before you begin

Follow these guidelines to prevent nuclease contamination

- Wear laboratory gloves for this protocol. Gloves protect you from the reagents and protect the nucleic acid from nucleases that are present on skin.
- Use nucleic acid-free pipette tips to handle the reagents, and avoid putting used tips into the reagent containers.
- Clean lab benches and pipettes with a nuclease decontamination solution. For example:

Reagent	Source
RNase*Zap*™ RNase Decontamination Solution	AM9780
DNA*Zap*™ PCR DNA Degradation Solutions	AM9890

Plate shaker compatibility and settings

Several procedures in this protocol require a plate shaker. When performing these procedures, we used a Barnstead/Lab-Line Titer Plate Shaker with the following settings:

- Moderate shaker speed — Settings 6 to 7
- Vigorous shaker speed — Settings 8 to 10

The settings above are based on a range of 1 to 10; setting 10 is the maximum setting. If you are using a different plate shaker, determine the maximum setting as follows:

Sample plate type	Determine the maximum setting
96 Deep Well Plates	1. Ensure that the 96 Deep Well Plate fits securely on your shaker. 2. Add 1 mL of water to each well of the 96 Deep Well Plate, then cover the plate with the Aluminum Adhesive Plate Sealer. 3. Determine the maximum setting that you can use on your shaker without any of the water splashing onto the Aluminum Adhesive Plate Sealer.
96 Standard Plates	1. Ensure that the 96 Standard Plate fits securely on your shaker. 2. Add 200 µL of water to each well of the 96 Deep Well Plate, then cover the plate with the Aluminum Adhesive Plate Sealer. 3. Determine the maximum setting that you can use on your shaker without any of the water splashing onto the Aluminum Adhesive Plate Sealer.

Before first use of the kit

Prepare Wash Solution 1

1. Add 125 mL of 100% isopropanol to the bottle of Wash Solution 1 Concentrate.
2. Mix well by inverting at least 5 times.
3. Mark the bottle label to indicate that 100% isopropanol was added.

Note: The resulting mixture is referred to as *Wash Solution 1* in this protocol.

Prepare Wash Solution 2

1. Add 232 mL of 100% ethanol to *each* bottle of Wash Solution 2 Concentrate.

Note: The 5X MagMAX™ Pathogen RNA/DNA Kit contains two bottles of Wash Solution 2 Concentrate.

2. Mix well by inverting at least 5 times.
3. Mark each bottle label to indicate that ethanol was added.

Note: The resulting mixture is referred to as *Wash Solution 2* in this protocol.

Low-cell-content samples

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 the low-cell-content samples

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 "Whole blood samples" on page 17).

- Plasma
- Swabs (nasal, tracheal, and cloacal)
- Ear notches

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 96–well instrument" on page 14 or "Process samples on the 24–well instrument" on page 16. If centrifugation is performed, the bacterial samples will be lost, as the bacteria will be pelleted with the other waste.

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

Proceed to "Process samples on the 96–well instrument" on page 14 or "Process samples on the 24–well instrument" on page 16.

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 96–well instrument" on page 14 or "Process samples on the 24–well instrument" on page 16.

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 96–well instrument” on page 14 or “Process samples on the 24–well instrument” on page 16.

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 96–well instrument” on page 14 or “Process samples on the 24–well instrument” on page 16.

Process samples on the 96–well instrument

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 a 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 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 96 Standard Plates.
   - For 100-, 200-, or 300-µL sample volumes, add 450 µL of prepared Wash Solution 2 to two 96 Deep Well Plates.

5. Prepare the elution plate by adding 90 µL of Elution Buffer to one 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 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	96 Deep Well Plate	Bead Mix	20 µL
			Sample	50 µL
			Lysis/ Binding Solution	130 µL
First Wash 1	2	96 Standard Plate	Wash Solution 1	150 µL
Second Wash 1	3	96 Standard Plate	Wash Solution 1	150 µL
First Wash 2	4	96 Standard Plate	Wash Solution 2	150 µL
Second Wash 2	5	96 Standard Plate	Wash Solution 2	150 µL
Elution	6	96 Standard Plate	Elution Buffer	90 µL
Tip comb plate	7	96 Standard Plate	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	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	96 Deep Well Plate	Wash Solution 1	300 µL	300 µL	300 µL
Second Wash 1	3	96 Deep Well Plate	Wash Solution 1	300 µL	300 µL	300 µL
First Wash 2	4	96 Deep Well Plate	Wash Solution 2	450 µL	450 µL	450 µL
Second Wash 2	5	96 Deep Well Plate	Wash Solution 2	450 µL	450 µL	450 µL
Elution	6	96 Standard Plate	Elution Buffer	90 µL	90 µL	90 µL
Tip comb plate	7	96 Standard Plate	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 24–well instrument

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

1. Select the 24 Deep Well processor script.
2. Insert the 24 Tip Combs into the instrument head.
3. Add 150 µL of prepared Wash Solution 1 to rows B and C of the 24 Deep Well 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 24 Deep Well Plate onto the processor, then start the 24 Deep Well 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.

Whole blood samples

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	200 µL
Carrier RNA (µg/µL)	2 µL
(Optional)[1] Xeno™ RNA or Xeno™ DNA	2 µL
100% Isopropanol	200 µL
Total volume for 1 reaction	**404 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 whole blood samples

You can use the MagMAX™ Pathogen RNA/DNA Kit to purify nucleic acid from 100 µL of whole blood samples.

1. Mix blood by inverting the blood samples at least 5 times.
2. Pulse spin for 1 second to collect the blood at the tube bottom.

Proceed to “Process samples on the 96–well instrument” on page 18.

Process samples on the 96–well instrument

For whole blood samples, we determined that 100 µL is the optimal input volume. The 96 Deep Well Plates are required for the volume that is used with this workflow

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 • KingFisher™ Apex Purification System
MagMAX™_Pathogen_High_Vol	• KingFisher™ Flex Magnetic Particle Processor

2. Prepare the tip comb plate: Place an 96 Deep Well Tip Comb in one 96 Standard Plate.
3. Prepare the Wash Solution 1 plates, by adding 300 µL of prepared Wash Solution 1 to two 96 Deep Well Plates.
4. Prepare the Wash Solution 2 plates, by adding 450 µL of prepared Wash Solution 2 to two 96 Deep Well Plates.
5. Prepare the elution plate, by adding 90 µL of Elution Buffer to one 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 96 processor and you have started the script.

   a. Add 20 µL of prepared Bead Mix to one 96 Deep Well Plate.
   b. Add 100 µL of prepared sample to the plate.
   c. Using a plate shaker, shake at moderate speed for 1 minute (see “Plate shaker compatibility and settings” on page 10 for shaker settings).

      Note: To avoid cross-contamination, do not pipet up and down.

   d. Add 400 µL of prepared Lysis/Binding Solution to the plate.

e. Using a plate shaker, shake at moderate speed for 1 minute.

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

Table 4 Processing plate setup : Whole blood (deep-well head configuration)

Plate ID	Plate position	Plate type	Reagent	Volume per well
Sample plate	1	96 Deep Well Plate	Bead Mix	20 µL
			Sample	100 µL
			Lysis/Binding Solution	400 µL
First Wash 1	2	96 Deep Well Plate	Wash Solution 1	300 µL
Second Wash 1	3	96 Deep Well Plate	Wash Solution 1	300 µL
First Wash 2	4	96 Deep Well Plate	Wash Solution 2	450 µL
Second Wash 2	5	96 Deep Well Plate	Wash Solution 2	450 µL
Elution	6	96 Standard Plate	Elution Buffer	90 µL
Tip comb plate	7	96 Standard Plate	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.

Semen samples

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	150 µL
Carrier RNA (µg/µL)	1 µL
(Optional)[1] Xeno™ RNA or Xeno™ DNA	2 µL
Total volume for 1 reaction	**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.

Prepare the semen samples

You can use the MagMAX™ Pathogen RNA/DNA Kit to purify nucleic acid from 115 µL of clarified semen lysate.

Select the appropriate preparation procedure:

- "Prepare the lysate using microcentrifuge tubes" on page 21 — Recommended for up to 24 samples
- "Prepare the lysate using plates" on page 21 — 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 semen vigorously (maximum setting) for 15 seconds.
3. Add 100 µL of the vortexed semen 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 96–well instrument" on page 22 or "Process samples on the 24–well instrument" on page 23.

Prepare the lysate using plates

This method is recommended for more than 24 samples.

1. Using a multichannel pipette, add 150 µL of the prepared Lysis/Binding Solution to each reaction well of a 96 Deep Well Plate.
2. Vortex the semen vigorously (maximum setting) for 15 seconds.
3. Add 100 µL of the vortexed semen 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 "Plate shaker compatibility and settings" on page 10 for shaker settings).
6. Centrifuge at ≥2500 *x g* (maximum setting) for 5 minutes to clarify the lysate.

Proceed to "Process samples on the 96–well instrument" on page 22 or "Process samples on the 24–well instrument" on page 23.

Process samples on the 96–well instrument

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 • KingFisher™ Apex Purification System
MagMAX™_Pathogen_Stnd_Vol	• KingFisher™ Flex Magnetic Particle Processor

2. Prepare the tip comb plate: Place an 96 Deep Well Tip Comb in one 96 Standard Plate.
3. Prepare the Wash Solution 1 plates, by adding 150 µL of prepared Wash Solution 1 to two 96 Standard Plates.
4. Prepare the Wash Solution 2 plates, 150 µL of prepared Wash Solution 2 to two 96 Standard Plates.
5. Prepare the elution plate, by adding 90 µL of Elution Buffer to one 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 96 processor and you have started the script.

   a. Add 20 µL of prepared Bead Mix to one 96 Deep Well Plate.
   b. Add 115 µL of prepared sample (clarified lysate) to the plate.
   c. Add 65 µL of 100% isopropanol to the plate.
7. Immediately start the 96 processor script, then load the plates onto the processor as directed.

Table 5 Processing plate setup: Semen (deep-well head configuration)

Plate ID	Plate position	Plate type	Reagent	Volume per well
Sample plate	1	96 Deep Well Plate	Bead Mix	20 µL
			Sample (clarified lysate)	115 µL
			100% Isopropanol	65 µL
First Wash 1	2	96 Standard Plate	Wash Solution 1	150 µL
Second Wash 1	3	96 Standard Plate	Wash Solution 1	150 µL

Table 5 Processing plate setup: Semen (deep-well head configuration) *(continued)*

Plate ID	Plate position	Plate type	Reagent	Volume per well
First Wash 2	4	96 Standard Plate	Wash Solution 2	150 µL
Second Wash 2	5	96 Standard Plate	Wash Solution 2	150 µL
Elution	6	96 Standard Plate	Elution Buffer	90 µL
Tip comb plate	7	96 Standard Plate	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 24–well instrument

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

1. Select the 24 Deep Well processor script.
2. Insert the 24 Tip Combs into the instrument head.
3. Add 150 µL of prepared Wash Solution 1 to rows B and C of the 24 Deep Well 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 24 Deep Well Plate onto the processor, then start the 24 Deep Well Plate processor script.

Table 6 Processing plate setup: Semen

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

Table 6 Processing plate setup: Semen *(continued)*

Row	Reagent	Volume per well
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.

Oral fluid samples

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 oral fluid samples

You can use the MagMAX™ Pathogen RNA/DNA Kit to purify nucleic acid from 600 µL of clarified oral fluid lysate.

Select the appropriate preparation procedure:

- "Prepare the lysate using microcentrifuge tubes" on page 26 — Recommended for up to 24 samples
- "Prepare the lysate using plates" on page 26 — 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 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 96–well instrument” on page 27.

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 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 “Plate shaker compatibility and settings” on page 10 for shaker settings).
6. Centrifuge at >2500 *x g* (maximum setting) for 5 minutes to clarify the lysate.

Proceed to “Process samples on the 96–well instrument” on page 27.

Process samples on the 96–well instrument

For oral fluid samples, we determined that 300 µL is the optimal input volume. The 96 Deep Well Plates are required for the volume that is used with this workflow.

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 • KingFisher™ Apex Purification System
MagMAX™_Pathogen_High_Vol	• KingFisher™ Flex Magnetic Particle Processor

2. Prepare the tip comb plate: Place an 96 Deep Well Tip Comb in one 96 Standard Plate.
3. Prepare the Wash Solution 1 plates, by adding 300 µL of prepared Wash Solution 1 to two 96 Deep Well Plates.
4. Prepare the Wash Solution 2 plates, by adding 450 µL of prepared Wash Solution 2 to two 96 Deep Well Plates.
5. Prepare the elution plate, by adding 90 µL of Elution Buffer to one 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 96 processor and you have started the script.

   a. Add 20 µL of prepared Bead Mix to one 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 96 processor script, then load the plates onto the processor as directed.

Table 7 Processing plate setup: Oral fluid (deep-well head configuration)

Plate ID	Plate position	Plate type	Reagent	Volume per well
Sample Plate	1	96 Deep Well Plate	Bead Mix	20 µL
			Sample (clarified lysate)	600 µL
			100% Isopropanol	350 µL
First Wash 1	2	96 Deep Well Plate	Wash Solution 1	300 µL
Second Wash 1	3	96 Deep Well Plate	Wash Solution 1	300 µL
First Wash 2	4	96 Deep Well Plate	Wash Solution 2	450 µL
Second Wash 2	5	96 Deep Well Plate	Wash Solution 2	450 µL
Elution	6	96 Standard Plate	Elution Buffer	90 µL
Tip comb plate	7	96 Standard Plate	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.

Fecal samples

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 the fecal samples

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

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 30 — Recommended for up to 24 samples
- "Prepare the lysate using plates" on page 31 — 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 96–well instrument" on page 31 or "Process samples on the 24–well instrument" on page 33.

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 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 “Plate shaker compatibility and settings” on page 10 for shaker settings).
5. Centrifuge at ≥2500 *x g* (maximum setting) for 5 minutes to clarify the lysate.

Proceed to “Process samples on the 96–well instrument” on page 31 or “Process samples on the 24–well instrument” on page 33.

Process samples on the 96–well instrument

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 • KingFisher™ Apex Purification System
	MagMAX™_Pathogen_Stnd_Vol	• KingFisher™ Flex Magnetic Particle Processor
400-µL clarified lysate	4462359_DW_HV	• KingFisher™ Flex Magnetic Particle Processor • KingFisher™ Apex Purification System
	MagMAX™_Pathogen_High_Vol	• KingFisher™ Flex Magnetic Particle Processor

2. Prepare the tip comb plate: Place an 96 Deep Well Tip Comb in one 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 96 Standard Plates.
   - For 400 µL of clarified lysate, add 300 µL of prepared Wash Solution 1 to two 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 96 Standard Plates.
   - For 400 µL of clarified lysate, add 450 µL of prepared Wash Solution 2 to two 96 Deep Well Plates.
5. Prepare the elution plate by adding 90 µL of Elution Buffer to one 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 96 processor and you have started the script.

   a. Add 20 µL of prepared Bead Mix to one 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 96 processor script, then load the plates onto the processor as directed.

Table 8 Plate processing setup: Fecal 115 µL (deep-well head configuration)

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

Table 9 Plate processing setup: Fecal 400 µL (deep-well head configuration)

Plate ID	Plate position	Plate type	Reagent	Volume per well
Sample plate	1	96 Deep Well Plate	Bead Mix	20 µL
			Sample (clarified lysate)	400 µL
			100% Isopropanol	350 µL
First Wash 1	2	96 Deep Well Plate	Wash Solution 1	300 µL
Second Wash 1	3	96 Deep Well Plate	Wash Solution 1	300 µL
First Wash 2	4	96 Deep Well Plate	Wash Solution 2	450 µL
Second Wash 2	5	96 Deep Well Plate	Wash Solution 2	450 µL
Elution	6	96 Standard Plate	Elution Buffer	90 µL
Tip comb plate	7	96 Standard Plate	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 24–well instrument

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

1. Select the 24 Deep Well processor script.
2. Insert the 24 Tip Combs into the instrument head.
3. Add 150 µL of prepared Wash Solution 1 to rows B and C of the 24 Deep Well 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 24 Deep Well Plate onto the processor, then start the 24 Deep Well Plate processor script.

Table 10 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.

InPouch™ TF culture samples

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.

Prepare the InPouch™ TF culture

You can use the MagMAX™ Pathogen RNA/DNA Kit to purify nucleic acid from Biomed Diagnostics InPouch™ TF *(Tritrichomonas foetus)* culture. You can use:

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

Prepare the InPouch™ TF culture sample

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 "Preparation the clarified lysate sample" on page 36.

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 immediately proceed to "Process InPouch™ TF culture samples on the 96–well instrument" on page 38.

Preparation the clarified lysate sample

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 "Prepare the InPouch™ TF culture sample" on page 36.

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 37 — Recommended for up to 24 samples
- "Prepare the lysate using plates" on page 37 — 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.

Proceed to one of the following:

- “Process InPouch™ TF culture samples on the 96–well instrument” on page 38
- “Process clarified lysate samples on the 96–well instrument” on page 39
- “Process clarified lysate samples on the 24–well instrument” on page 40

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 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 “Plate shaker compatibility and settings” on page 10 for shaker settings).
6. Centrifuge at ≥2500 *x g* (maximum setting) for 5 minutes to clarify the lysate.

Proceed to one of the following:

- “Process InPouch™ TF culture samples on the 96–well instrument” on page 38
- “Process clarified lysate samples on the 96–well instrument” on page 39
- “Process clarified lysate samples on the 24–well instrument” on page 40

Process InPouch™ TF culture samples on the 96–well instrument

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 • KingFisher™ Apex Purification System
MagMAX™_Pathogen_High_Vol	• KingFisher™ Flex Magnetic Particle Processor

2. Prepare the tip comb plate: Place an 96 Deep Well Tip Comb in one 96 Standard Plate.
3. Prepare the Wash Solution 1 plates, by adding 300 µL of prepared Wash Solution 1 to two 96 Deep Well Plates.
4. Prepare the Wash Solution 2 plates, by adding 450 µL of prepared Wash Solution 2 to two 96 Deep Well Plates.
5. Prepare the elution plate, by adding 90 µL of Elution Buffer to one 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 96 processor and you have started the script.

   a. Add 20 µL of prepared Bead Mix to one 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 96 processor script, then load the plates onto the processor as directed.

Table 11 Processing plate setup: InPouch™ TF culture (deep-well head configuration)

Plate ID	Plate position	Plate type	Reagent	Volume per well
Sample plate	1	96 Deep Well Plate	Bead Mix	20 µL
			Sample	300 µL
			Lysis/Binding Solution	700 µL
First Wash 1	2	96 Deep Well Plate	Wash Solution 1	300 µL
Second Wash 1	3	96 Deep Well Plate	Wash Solution 1	300 µL

Table 11 Processing plate setup: InPouch TF culture (deep-well head configuration) *(continued)*

Plate ID	Plate position	Plate type	Reagent	Volume per well
First Wash 1	4	96 Deep Well Plate	Wash Solution 2	450 µL
Second Wash 2	5	96 Deep Well Plate	Wash Solution 2	450 µL
Elution	6	96 Standard Plate	Elution Buffer	90 µL
Tip comb plate	7	96 Standard Plate	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 clarified lysate samples on the 96–well instrument

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 • KingFisher™ Apex Purification System
MagMAX™_Pathogen_Stnd_Vol	• KingFisher™ Flex Magnetic Particle Processor

2. Prepare the tip comb plate: Place an 96 Deep Well Tip Comb in one 96 Standard Plate.
3. Prepare the Wash Solution 1 plates, by adding 150 µL of prepared Wash Solution 1 to two 96 Standard Plates.
4. Prepare the Wash Solution 2 plates, by adding 150 µL of prepared Wash Solution 2 to two 96 Standard Plates.
5. Prepare the elution plate, by adding 90 µL of Elution Buffer to one 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 96 processor and you have started the script.

   a. Add 20 µL of prepared Bead Mix to one 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 96 processor script, then load the plates onto the processor as directed.

Table 12 Processing plate setup: clarified lysate (deep-well head configuration)

Plate ID	Plate position	Plate type	Reagent	Volume per well
Sample plate	1	96 Deep Well Plate	Bead Mix	20 µL
			Sample (clarified lysate)	115 µL
			100% Isopropanol	65 µL
First Wash 1	2	96 Standard Plate	Wash Solution 1	150 µL
Second Wash 1	3	96 Standard Plate	Wash Solution 1	150 µL
First Wash 2	4	96 Standard Plate	Wash Solution 2	150 µL
Second Wash 2	5	96 Standard Plate	Wash Solution 2	150 µL
Elution	6	96 Standard Plate	Elution Buffer	90 µL
Tip comb plate	7	96 Standard Plate	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 clarified lysate samples on the 24–well instrument

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

1. Select the 24 Deep Well processor script.
2. Insert the 24 Tip Combs into the instrument head.
3. Add 150 µL of prepared Wash Solution 1 to rows B and C of the 24 Deep Well 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 24 Deep Well Plate onto the processor, then start the 24 Deep Well processor script.

Table 13 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.

Troubleshooting

Analyzing RNA and DNA

Detect RNA by real-time RT-PCR and detect DNA by real-time PCR

This kit is designed for purification of RNA for RT-PCR amplification, and purification of DNA for PCR. Quantitative real-time RT-PCR/PCR is a powerful method for RNA/DNA detection and is the recommended analysis tool.

Quantitate carrier RNA recovered

The Viral RNA and DNA recovered from most samples will be present in very limited amounts; the majority of nucleic acid in the purified sample will be the Carrier RNA that was added to the Lysis/Binding Solution. Viral RNA and DNA recovery is heavily dependent upon sample type (for example, plasma vs. swab samples). With most sample types, 50% to 75% of the Carrier RNA should be recovered. Using the recommended volume of prepared Lysis/Binding Solution, each sample will contain approximately 1 µg of Carrier RNA; therefore >5 ng/µL of Viral RNA or DNA should be recovered.

Quantitate the amount of Carrier RNA by UV absorbance at 260 nm (A_{260}). If you are using a NanoDrop™ 1000 Spectrophotometer, 1.5 µL of nucleic acid solution can be measured without dilution.

Alternatively, the Carrier RNA concentration can be determined by diluting an aliquot of the preparation in TE (10 mM Tris-HCl, pH 8; 1 mM EDTA) and reading the absorbance in a traditional spectrophotometer at 260 nm. Find the concentration in µg/mL by multiplying the A_{260} by the dilution factor and the extinction coefficient:

1 A_{260}= 40 µg RNA/mL

Observation	Possible cause	Recommended action
Poor or no RNA or DNA signal (that is_ the Ct value is higher than expected)	There are inhibitors in the recovered nucleic acid. With most samples, this Protocol yields very pure nucleic acid; however, with samples that contain excessively high amounts of reaction inhibitors, enough may be carried over to affect RT-PCR.	Reduce the amount of RNA used in the RT-PCR or the amount of DNA used in the PCR. Try diluting the eluted nucleic acid 10-fold and repeating the RT-PCR or PCR. If a signal is observed using the diluted sample, inhibitors may be present in the eluted nucleic acid.
		Perform UV absorbance at 260 and 280 nm to determine if there is protein contamination in the sample. Proteins have an absorbance peak at ~280 nm, whereas nucleic acids have an absorbance peak at ~260 nm. The ratio of A_{260}/A_{280} should be ~2.0 for pure nucleic acid isolated from cell-free samples with the MagMAX™ Pathogen RNA/DNA Kit. An A_{260}/A_{280} ratio below indicates protein carryover.
Lower-than-expected carrier RNA recovery	Poor recovery of the Carrier RNA (>5 ng/µL) could indicate a problem with the nucleic acid purification process.	See "Well-to-well variation in RNA/DNA yield" on page 44 for suggestions that may help with nucleic acid recovery. If these suggestions do not improve Carrier RNA recovery, the procedure may require further optimization for use with different sample types. Contact Technical Support for more information on how to optimize the kit for use with various sample types.

Observation	Possible cause	Recommended action
Well-to-well variation in RNA/DNA yield	The Nucleic Acid Binding Beads were not fully resuspended/dispersed.	In general, the beads will disperse more easily when the temperature of the mixture is >20°C. Be sure that you: • Fully resuspend the Bead Mix before adding it to the plate. • Fully resuspend the beads in the Elution Buffer. Fully resuspended beads produce a homogenous brown solution. If the solution is clear, with brown clumps, it means that the beads are not fully resuspended. Preheat the Elution Buffer to 60 to 65°C just before use to help resuspend the beads. • Do not overdry the beads before eluting the RNA/DNA. Overdrying may make the beads more difficult to resuspend. If the beads are inadvertently overdried, increase the mixing time 10 minutes during the elution step to allow the beads to rehydrate.
	The Nucleic Acid Binding Beads were unintentionally lost. Because the principle of this procedure is to immobilize nucleic acids on the Nucleic Acid Binding Beads, any loss of beads during the procedure will result in loss of RNA/DNA.	Avoid aspirating the beads when removing supernatant from the captured beads. To determine whether beads have been inadvertently aspirated with the supernatant, it may be helpful to collect all supernatants (except the final RNA-containing supernatant) in a single container. If the collected supernatant is light brown, beads are in the supernatant. To prevent aspiration of the beads in subsequent experiments: • Use sufficient magnetic capture time. • Aspirate the supernatant slowly. • Keep pipette tip openings away from the captured beads when aspirating the supernatant.
Good recovery of carrier RNA_ but the sample RNA or DNA cannot be detected	If the Carrier RNA was recovered at expected levels (>5 ng/µL of RNA), but the sample RNA or DNA cannot be detected using a proven RT-PCR or PCR assay system, this would suggest the absence of the pathogen in the original sample, or problems with the RT-PCR or PCR.	Consider the recommendation in the previous section for diluting your sample to minimize the effects of inhibitors.

Observation	Possible cause	Recommended action
The eluate is light brown in color	The Nucleic Acid Binding Beads were carried over into the eluate.	A small quantity of beads in the sample does not inhibit RT reactions or RT-PCR: See “Well-to-well variation in RNA/DNA yield” on page 44 ("Possible cause: The Nucleic Acid Binding Beads were unintentionally lost") for suggestions on avoiding bead carryover. To remove the beads from RNA samples, place the plate on a magnetic stand to capture the beads for ~1 minute, then transfer the nucleic acid solution to a new nuclease-free plate or tubes.

B

Manual methods

This appendix provides manual procedures for purifying nucleic acid (RNA and DNA) from the following sample types:

- Low-cell-content samples:
  - Serum
  - Plasma
  - Swabs (nasal, tracheal, and cloacal)
  - Ear notches
- Semen
- Feces
- Biomed Diagnostics InPouch TF *(Tritrichomonas foetus)* culture

User-supplied materials

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Reagents

Reagent	Source	Sample type			
		Low-cell-content	Semen	Feces	InPouch™ TF culture
100% Isopropanol, ACS reagent grade or equivalent	MLS		X	X	X

General laboratory equipment

Item	Source	Sample type			
		Low-cell-content	Semen	Feces	InPouch™ TF culture
Disposable gloves	MLS	X	X	X	X
Pipette tips[1]					
P1000	MLS	X	X	X	X
Large-bore	MLS		X	X	

(continued)

Item	Source	Sample type			
		Low-cell-content	Semen	Feces	InPouch™ TF culture
Pipettes					
Single- and multichannel pipettes	MLS	X	X	X	X
(Optional) Disposable serological pipettes (25- to 50-mL), or equivalent, and a pipetting device for the serological pipettes	MLS	X	X	X	X
Repeater pipettes	MLS		X	X	X
Disposable pipettes, sterile	MLS				X
96-well processing plate					
96-Well Microplate, U-Bottom Polystyrene	Phenix MPU-8117, or equivalent	X	X	X	X
Untreated Lids for 96-Well Microplates, Clear	Phenix MPE-8019, or equivalent	X	X	X	X
MicroAmp™ Clear Adhesive Film (for protecting unused wells of the 96-well processing plate)	4306311	X	X	X	X
Magnetic stand, one of the following					
Magnetic Stand-96	AM10027	X	X	X	X
96 well Magnetic-Ring Stand	AM10050	X	X	X	X
Barnstead/Lab-Line Titer Plate Shaker	MLS	X	X	X	X
Hybridization oven or dry incubator	MLS	X	X	X	X

[1] Pipette tips should be aerosol-resistant and nuclease-free.

Before you begin

Follow these guidelines to prevent nuclease contamination

- Wear laboratory gloves for this protocol. Gloves protect you from the reagents and protect the nucleic acid from nucleases that are present on skin.
- Use nucleic acid-free pipette tips to handle the reagents, and avoid putting used tips into the reagent containers.

- Clean lab benches and pipettes with a nuclease decontamination solution. For example:

Reagent	Source
RNase*Zap*™ RNase Decontamination Solution	AM9780
DNA*Zap*™ PCR DNA Degradation Solutions	AM9890

Determine plate shaker compatibility and settings

Several procedures in this protocol require a plate shaker. When performing these procedures, we used a Barnstead/Lab-Line Titer Plate Shaker with the following settings:

- Moderate shaker speed — Settings 6 to 7
- Vigorous shaker speed — Settings 8 to 10

The settings above are based on a range of 1 to 10; setting 10 is the maximum setting. If you are using a different plate shaker, determine the maximum setting as follows:

1. Verify that the 96-well processing plate fits securely on your shaker.
2. Add 250 µL of water to each well of the 96-well processing plate.
3. Determine the maximum setting that you can use on your shaker without any of the water spilling.

Prepare the reagents

Prepare Wash Solutions 1 and 2, Lysis/Binding Solution, and Bead Mix

Prepare the reagents according to the procedures referenced in the table below.

Reagent	See page...
Wash Solution 1	"Prepare Wash Solution 1" on page 11
Wash Solution 2	"Prepare Wash Solution 2" on page 11
Lysis/Binding Solution	• Low cell content samples: "Prepare the Lysis/Binding Solution" on page 12 • Whole blood samples: "Prepare Lysis/Binding solution" on page 17 • Semen samples: "Prepare Lysis/Binding Solution" on page 20 • Oral fluid samples: "Prepare Lysis/Binding Solution" on page 25 • Fecal samples: "Prepare Lysis/Binding Solution" on page 29 • InPouch TF culture: "Prepare Lysis/Binding Solution" on page 35
Bead Mix	"Prepare the Bead Mix" on page 12 [1]

[1] Same for all sample types.

Prepare the Elution Buffer

Each purification reaction requires 90 µL of heated Elution Buffer. Prepare the Elution Buffer at least 10 minutes before performing the final nucleic acid elution step (see “Elute the nucleic acid” on page 51).

1. Set a hybridization oven or dry incubator to 65°C.
2. Heat the required volume of Elution Buffer at 65°C for at least 10 minutes.

Prepare the samples

Prepare your samples according to the appropriate procedure:

Sample type	See page...
Low-cell-content	“Prepare the low-cell-content samples” on page 13
Semen	“Prepare the semen samples” on page 20
Feces	“Prepare the fecal samples” on page 30
InPouch™ TF culture	“Prepare the InPouch™ TF culture” on page 35

Purify the nucleic acid

Purification procedure guidelines

- Perform the purification procedure at room temperature (18 to 25°C), unless otherwise stated.
- When aspirating, be careful not to dislodge the Nucleic Acid Binding Beads from the magnet.
- The capture time of the Nucleic Acid Binding Beads depends on the magnetic stand that you use.
- When capturing the Nucleic Acid Binding Beads on the magnetic stand, you can remove the supernatant after the solution becomes clear and the beads form a pellet at the magnet.

Lyse the sample, bind the nucleic acid, then capture the beads

Note: Only the low-cell-content samples are lysed during this procedure. Semen samples, fecal samples, and InPouch™ TF culture are lysed during sample preparation (see "Prepare the semen samples" on page 20, "Prepare the fecal samples" on page 30, and "Prepare the InPouch™ TF culture" on page 35).

1. To each reaction well of a 96-well processing plate, add the following components *in the order indicated*:

Component	Sample type			
	Low-cell-content	Semen	Feces	InPouch™ TF culture
Prepared Bead Mix	20 µL	20 µL	20 µL	20 µL
Prepared sample	50 µL	115 µL	115 µL	115 µL
Prepared Lysis/ Binding Solution	130 µL	—	—	—
100% Isopropanol	—	65 µL	65 µL	65 µL

2. Using a plate shaker, shake at moderate speed for 3 minutes (see "Plate shaker compatibility and settings" on page 10 for shaker settings).
3. Use a magnetic stand to capture the beads. The capture time is 3 minutes.
4. Carefully aspirate, then discard all of the supernatant without disturbing the beads.
5. Remove the plate from the magnetic stand.

Note: It is critical that you remove the plate from the magnetic stand before you perform the next step.

Wash twice with Wash Solution 1

1. Add 150 µL of prepared Wash Solution 1 to each reaction well.
2. Using a plate shaker, shake at moderate speed for 1 minute (see "Plate shaker compatibility and settings" on page 10 for shaker settings).

Note: Beads may be evenly dispersed or may clump. Variable dispersion is expected and does not significantly affect results.

3. Use a magnetic stand to capture the beads. The capture time is ~1 minute. When the sample mixture is transparent, the beads have been captured.
4. Carefully aspirate and discard all supernatant without disturbing the beads.

5. Remove the plate from the magnetic stand.

   Note: It is critical that you remove the plate from the magnetic stand before the next step.

6. Repeat steps step 1 to step 5 one time.

Wash twice with Wash Solution 2

1. Add 150 µL of prepared Wash Solution 2 to each reaction well.
2. Using a plate shaker, shake at moderate speed for 1 minute (see “Plate shaker compatibility and settings” on page 10 for shaker settings).

   Note: Beads may be evenly dispersed or may clump. Variable dispersion is expected and does not significantly affect results.

3. Use a magnetic stand to capture the beads. The capture time is ~1 minute. When the sample mixture is transparent, the beads have been captured.
4. Carefully aspirate and discard all supernatant without disturbing the beads.
5. Remove the plate from the magnetic stand.

   Note: It is critical that you remove the plate from the magnetic stand before the next step.

6. Repeat steps step 1 to step 5 above one time.

Dry the beads

1. Using a plate shaker, with the plate lid removed, shake the plate at vigorous speed for 2 minutes (see “Plate shaker compatibility and settings” on page 10 for shaker settings).
2. Inspect the plate. Aspirate, then discard any remaining Wash Solution 2.

Elute the nucleic acid

1. Add 90 µL of 65°C Elution Buffer (from “Prepare the Elution Buffer” on page 49) to each reaction well.
2. Using a plate shaker, shake at vigorous speed for 3 minutes (see “Plate shaker compatibility and settings” on page 10 for shaker settings).

   After 3 minutes, the sample solution should be brown, indicating complete bead suspension.

   Note: Complete bead suspension is critical for high and consistent nucleic acid recovery.

3. *(Optional)* Resuspend any remaining clumped beads.
   a. Using a multichannel pipette, gently pipet up and down 6 to 8 times to resuspend the beads. To avoid cross-contamination, be careful not to splash liquid while pipetting up and down and change the pipette tips between wells.

b. Using a plate shaker, shake at vigorous speed for 1 minute.

4. Use a magnetic stand to capture the beads. The capture time is ~2 minutes.

5. Being careful not to disturb the beads, transfer 85 µL of supernatant to a clean reaction plate. **Do not discard the supernatant; the purified nucleic acid is in the supernatant.**

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.

C

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311