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MagMAX™ Pathogen RNA/DNA Kit

Protocol

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About This Guide

Safety information

For general safety information, see this section and Appendix B, "Safety" on page 57.

Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

SDSs

The Safety Data Sheets (SDSs) for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining SDSs, see "SDSs" on page 58.

IMPORTANT! For the SDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

MagMAX™ Pathogen RNA/DNA Kit Protocol

Product information

Purpose of the product

The MagMAX™ Pathogen RNA/DNA Kit is designed for rapid purification of nucleic acid (RNA and DNA) from the following sample types:

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

Applications

You can use the MagMAX Pathogen RNA/DNA Kit for:

- High-throughput applications – Perform the RNA/DNA purifications on the MagMAX™ Express-96 Deep Well Magnetic Particle Processor (MME-96 processor) or the MagMAX™ Express Magnetic Particle Processor (MME-24 processor). **All MME-96 scripts (for ≥50-µL sample volumes) included in this protocol require the Deep Well Magnetic Head.**
- Low- and medium-throughput applications – Manually perform the RNA/DNA purifications in a 96-well processing plate to process up to 96 samples at once.

The table below lists the sample input volumes for each sample type.

Sample type	Sample input volume		
	MME-96 processor	MME-24 processor	Manual processing
Low-cell-content	50, 100, 200, and 300 µL	50 µL	50 µL
Semen†	115 µL	115 µL	115 µL
Feces†	115 and 400 µL	115 µL	115 µL
InPouch™ TF culture†	115 and 300‡ µL	115 µL	115 µL
Whole blood	100 µL	---	---
Oral fluid†	600 µL	---	---

† Clarified lysate volumes are listed.

‡ 300 µL of InPouch™ TF culture is a non-lysate protocol that requires the MagMAX™ Express-96 Deep Well Magnetic Particle Processor.

How the kit works

The MagMAX Pathogen RNA/DNA Kit works as follows:

1. Samples are disrupted in a guanidinium thiocyanate-based solution that rapidly releases RNA and DNA while simultaneously inactivating nucleases in the sample matrix.
2. Paramagnetic particles with a nucleic acid binding surface are added to the sample to bind nucleic acids.
3. The particles and nucleic acids are captured on magnets.
4. The particles are washed four times to remove proteins, contaminants (PCR inhibitors), and residual binding solution.
5. Nucleic acids are eluted in a small volume of elution buffer.

Note: This procedure recovers total nucleic acids, so if cells are present in the sample, cellular DNA/RNA will be recovered along with the pathogen DNA/RNA.

Kit contents and storage conditions

Upon receipt, store each component as indicated below.

Part number	Name	Kit contents		
		Component	Quantity	Storage conditions
4462359	5× MagMAX™ Pathogen RNA/DNA Kit	Lysis/Binding Solution Concentrate	240 mL	Room temperature
		Wash Solution 1 Concentrate	250 mL†	
		Wash Solution 2 Concentrate	2 × 58 mL‡	
		Elution Buffer	60 mL	
		Nucleic Acid Binding Beads	5.5 mL	4°C Do not freeze
		Carrier RNA	5 × 220 µL	–20°C
		Lysis ENHANCER	5.5 mL	

† Before using the Wash Solution 1 Concentrate, add 125 mL of 100% isopropanol (user-supplied). The final volume will be 375 mL.

‡ Before using the Wash Solution 2 Concentrate, add 232 mL of 100% ethanol (user-supplied) to *each* bottle of Wash Solution 2 Concentrate. The final volume in each bottle will be 290 mL.

User-supplied materials

Unless otherwise indicated, all items are available from major laboratory suppliers (MLS).

IMPORTANT! For the SDS (Safety Data Sheet) of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Reagents

Reagent	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					
Xeno™ RNA or Xeno™ DNA (If you are using VetMAX™-Plus Reagents)	Applied Biosystems (Part no. 4415327, 4415330, 4415328)	X	X	X	X	X	X
1× PBS, pH 7.4 (sterile)	MLS					X	X

Particle processors, plates, and combs

Item	Applied Biosystems part number
MagMAX™ Express-96 Deep Well Magnetic Particle Processor (for processing up to 96 samples; also referred to as the *MME-96 processor*)	4400079
MagMAX™ Express-96 Deep Well Plates	4388476
MagMAX™ Express-96 Standard Plates	4388475
MagMAX™ Express-96 Deep Well Tip Combs	4388487
MagMAX™ Express Magnetic Particle Processor (for processing up to 24 samples; also referred to as the *MME-24 processor*)	4400075
MagMAX™ Express Plates	4388474
MagMAX™ Express Tip Combs	4388452

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 – various, including:							
• p1000	MLS	X	X	X	X	X	X
• Large-bore	MLS		X	X		X	
Note: Pipette tips should be aerosol-resistant and nuclease-free.							
Pipettes:							
• Single- and multichannel pipettes	MLS	X	X	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	X	X
• Repeater pipettes	MLS		X	X	X	X	X
• Disposable pipettes, sterile	MLS				X		X
Microcentrifuge tubes:							
• 1.5-mL	MLS	X	X	X	X	X	X
• 2-mL	MLS	X	X	X		X	
Conical-Bottom Centrifuge Tubes, Polypropylene, 5-mL	VWR (Part no. 80076-634)						X
Microcentrifuge (capable of 16,000 × *g*)	Eppendorf (Part no. 5415D), or equivalent	X	X	X	X	X	X
Plate centrifuge, with tube adaptors	Eppendorf (Part no. 5804), or equivalent		X	X	X	X	X
Aluminum Adhesive Plate Sealer	VWR (Part no. 14230-062)		X	X	X	X	X
Barnstead/Lab-Line Titer Plate Shaker	VWR (Part no. 57019-600), or equivalent	X	X	X	X	X	X
Vortexer	MLS	X	X	X	X	X	X
Vortex adaptor	Ambion Vortex Adapter (Part no. AM10024), or equivalent	X	X	X	X	X	X

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*® surface decontamination solution	Ambion (Part no. AM9780)
DNA*Zap*™ surface decontamination spray	Ambion (Part no. 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:

Sample type	Determine the maximum setting
Oral fluid	1. Verify that the MME-96 Deep Well Plate fits securely on your shaker. 2. Add 750 µL of water to each well of the MME-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.
All other sample types	1. Verify that the MME-96 Deep Well Plate fits securely on your shaker. 2. Add 250 µL of water to each well of the MME-96 Deep Well Plate. 3. Determine the maximum setting that you can use on your shaker without any of the water spilling.

Prepare the reagents

When preparing the reagents:

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

Prepare Wash Solution 1

You can prepare Wash Solution 1 ahead of time; store at room temperature for up to 6 weeks.

1. Add 125 mL of 100% isopropanol to the bottle of Wash Solution 1 Concentrate.
2. Mix well by inverting 3 to 4 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

You can prepare Wash Solution 2 ahead of time; store at room temperature for up to 6 weeks.

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

   Note: The 5× MagMAX™ Pathogen RNA/DNA Kit contains two bottles of Wash Solution 2 Concentrate.
2. Mix well by inverting 3 to 4 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.

Prepare the Lysis/Binding Solution

Prepare the Lysis/Binding Solution as needed. Store at room temperature for up to 8 hours.

For low-cell-content samples and 300 µL of InPouch™ TF culture

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

Order to combine	Component	Starting sample volume			
		50 µL	100 µL	200 µL	300 µL
1	Lysis/Binding Solution Concentrate	65 µL	125 µL	250 µL	350 µL
2	Carrier RNA (µg/µL)	1 µL	2 µL	2 µL	2 µL
3	Xeno™ RNA or Xeno™ DNA (If you are using VetMAX™-Plus Reagents)	2 µL	2 µL	2 µL	2 µL
4	100% Isopropanol	65 µL	125 µL	250 µL	350 µL
Total volume for 1 reaction		**133 µL**	**254 µL**	**504 µL**	**704 µL**

2. Mix well by vortexing.

For whole blood samples

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

Order to combine	Component	Volume
1	Lysis/Binding Solution Concentrate	200 µL
2	Carrier RNA (µg/µL)	2 µL
3	Xeno™ RNA or Xeno™ DNA (If you are using VetMAX™-Plus Reagents)	2 µL
4	100% Isopropanol	200 µL
Total volume for 1 reaction		**404 µL**

2. Mix well by vortexing.

For all other sample types

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

Order to combine	Component	Volume			
		Semen	Oral fluid	Feces	InPouch™ TF culture
1	Lysis/Binding Solution Concentrate	150 µL	450 µL	500 µL	150 µL
2	Carrier RNA (µg/µL)	1 µL	2 µL	2 µL	1 µL
3	Xeno™ RNA or Xeno™ DNA (If you are using VetMAX™-Plus Reagents)	2 µL	2 µL	2 µL	2 µL
Total volume for 1 reaction		**153 µL**	**454 µL**	**504 µL**	**153 µL**

2. Mix well by vortexing.

Prepare the Bead Mix

Prepare the Bead Mix as needed. Store on ice for up to 4 hours.

1. Vortex the Nucleic Acid Binding Beads well.
2. On ice, combine the components 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.

Section 1 Low-Cell-Content Samples

This section covers:

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 amount of red blood cells, we recommend that you follow the whole blood procedure (see page 23).

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

Preparing swabs

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

Proceed to "Purify the nucleic acid" on page 19.

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 "Purify the nucleic acid" on page 19.

Preparing ear notches

Dry ear notches

1. Place each dry ear notch sample into separate 2-mL microcentrifuge tubes.
2. Add 1 mL of 1✕ 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 × *g* (maximum setting) for 30 seconds to collect the tube contents.

Proceed to "Purify the nucleic acid" on page 19.

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 "Purify the nucleic acid" on page 19.

Purify the nucleic acid

This section provides procedures for purifying nucleic acid on the 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 Appendix A on page 49.

MME-96 processor

IMPORTANT! 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 appropriate MME-96 script:
   - For 50-µL sample volumes, select **4462359_DW_50**.
   - For 100-, 200-, or 300-µL sample volumes, select **4462359_DW_HV**.
2. Prepare the tip comb plate: Place an MME-96 Deep Well Tip Comb in one MME-96 Standard Plate.
3. Add prepared Wash Solution 1:
   - For 50-µL sample volumes, add 150 µL to two MME-96 Standard Plates.
   - For 100-, 200-, or 300-µL sample volumes, add 300 µL to two MME-96 Deep Well Plates.
4. Add prepared Wash Solution 2:
   - For 50-µL sample volumes, add 150 µL to two MME-96 Standard Plates.
   - For 100-, 200-, or 300-µL sample volumes, add 450 µL to two MME-96 Deep Well Plates.
5. Add 90 µL of Elution Buffer to one MME-96 Standard Plate (not the tip comb plate).
6. Prepare the sample plate:

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

   a. Add 20 µL of prepared Bead Mix to one MME-96 Deep Well Plate.
   b. Add prepared sample to the plate according to Table 1 on page 20.
   c. Add prepared Lysis/Binding Solution to the plate according to Table 1 on page 20.
7. Immediately start the MME-96 processor script, then load the plates onto the processor as directed.

Table 1 MME-96 processor protocol for low-cell-content samples

Sample volume		**50 µL, 100 µL, 200 µL, or 300 µL**						
MME-96 script		**4462359_DW_50 (For 50-µL sample volumes)** **4462359_DW_HV (For 100-, 200-, or 300-µL sample volumes)**						
Machine configuration		**MME-96 Deep Well Magnetic Head**						
Tip comb		**MME-96 Deep Well Tip Comb**						
Plate position		**Reagent**	**Volume per well**				**MME-96 plate to use for each sample volume**	
Plate	**Description**						**50 µL**	**100, 200, 300 µL**
1	Sample plate	Bead Mix	20 µL	20 µL	20 µL	20 µL	Deep Well	Deep Well
		Sample	50 µL	100 µL	200 µL	300 µL		
		Lysis/Binding Solution	130 µL	250 µL	500 µL	700 µL		
2	First Wash 1	Wash Solution 1	150 µL	300 µL	300 µL	300 µL	Standard	Deep Well
3	Second Wash 1	Wash Solution 1	150 µL	300 µL	300 µL	300 µL	Standard	Deep Well
4	First Wash 2	Wash Solution 2	150 µL	450 µL	450 µL	450 µL	Standard	Deep Well
5	Second Wash 2	Wash Solution 2	150 µL	450 µL	450 µL	450 µL	Standard	Deep Well
6	Elution	Elution Buffer	90 µL	90 µL	90 µL	90 µL	Standard	Standard
7	Tip comb plate	MME-96 Deep Well Tip Comb in plate					Standard	Standard

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.

MME-24 processor

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 2 MME-24 processor protocol for low-cell-content samples

Sample volume		**50 µL**	
MME-24 script		**4462359**	
Plate to use		**MME-24 Plate**	
Tip comb		**MME-24 Tip Comb**	
Well position		**Reagent**	**Volume per well**
Row	**Description**		
Row A	Sample wells	Bead Mix	20 µL
		Sample	50 µL
		Lysis/Binding Solution	130 µL
Row B	First Wash 1	Wash Solution 1	150 µL
Row C	Second Wash 1	Wash Solution 1	150 µL
Row D	First Wash 2	Wash Solution 2	150 µL
Row E	Second Wash 2	Wash Solution 2	150 µL
Row F	Elution	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.

Section 2 Whole Blood Samples

This section covers:

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. Vortex the blood samples vigorously (maximum setting) for 30 seconds.
2. Pulse spin for 1 second to collect the blood at the tube bottom.

Proceed to "Purify the nucleic acid" (this page).

Purify the nucleic acid

This section provides procedures for purifying nucleic acid on the MagMAX™ Express-96 Deep Well Magnetic Particle Processor (MME-96 processor).

Note: For whole blood samples, we determined that 100 µL is the optimal input volume. Because Deep Well Plates are required for ≥100-µL volumes, we have not included MagMAX™ Express Magnetic Particle Processor (MME-24 processor) or manual purification procedures.

MME-96 processor

IMPORTANT! 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 **4462359_DW_HV** MME-96 script.
2. Prepare the tip comb plate: Place an MME-96 Deep Well Tip Comb in one MME-96 Standard Plate.
3. Add 300 µL of prepared Wash Solution 1 to two MME-96 Deep Well Plates.
4. Add 450 µL of prepared Wash Solution 2 to two MME-96 Deep Well Plates.
5. Add 90 µL of Elution Buffer to one MME-96 Standard Plate (not the tip comb plate).

6. Prepare the sample plate:

   IMPORTANT! 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 100 µL of prepared sample to the plate.
   c. Using a plate shaker, shake at moderate speed for 1 minute (see page 13 for shaker settings).

      IMPORTANT! 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 MME-96 processor script, then load the plates onto the processor as directed.

Table 3 MME-96 processor protocol for whole blood samples

Sample volume		**100 µL**		
MME-96 script		**4462359_DW_HV**		
Machine configuration		**MME-96 Deep Well Magnetic Head**		
Tip comb		**MME-96 Deep Well Tip Comb**		
Plate position		**Reagent**	**Volume per well**	**Plate to use**
Plate	**Description**			
1	Sample plate	Bead Mix	20 µL	MME-96 Deep Well Plate
		Sample	100 µL	
		Lysis/Binding Solution	400 µL	
2	First Wash 1	Wash Solution 1	300 µL	MME-96 Deep Well Plate
3	Second Wash 1	Wash Solution 1	300 µL	MME-96 Deep Well Plate
4	First Wash 2	Wash Solution 2	450 µL	MME-96 Deep Well Plate
5	Second Wash 2	Wash Solution 2	450 µL	MME-96 Deep Well Plate
6	Elution	Elution Buffer	90 µL	MME-96 Standard Plate
7	Tip comb plate	MME-96 Deep Well Tip Comb in plate		MME-96 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.

Section 3 Semen Samples

This section covers:

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" (this page) – Recommended for ≤24 samples
- "Prepare the lysate using plates" (this page) – Recommended for >24 samples

Prepare the lysate using microcentrifuge tubes

This method is recommended for ≤24 samples.

For each sample:

1. Using a repeater pipette, add 150 µL of the prepared Lysis/Binding Solution to a 1.5-mL microcentrifuge tube.
2. To ensure uniform sampling, vortex the semen vigorously (maximum setting) for 15 seconds, then proceed immediately to step 3 below.
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 × *g* (maximum setting) for 2 minutes to clarify the lysate.

Proceed to "Purify the nucleic acid" on page 26.

Prepare the lysate using plates

This method is recommended for >24 samples.

1. Using a multichannel pipette, add 150 µL of the prepared Lysis/Binding Solution to each reaction well of a MagMAX™ Express-96 Deep Well Plate.
2. To ensure uniform sampling, vortex the semen vigorously (maximum setting) for 15 seconds, then proceed immediately to step 3 below.
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 page 13 for shaker settings).
6. Centrifuge at ≥2500 × *g* (maximum setting) for 5 minutes to clarify the lysate.

Proceed to "Purify the nucleic acid" (this page).

Purify the nucleic acid

This section provides procedures for purifying nucleic acid on the 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 Appendix A on page 49.

MME-96 processor

IMPORTANT! 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 **4462359_DW_50** MME-96 script.
2. Prepare the tip comb plate: Place an MME-96 Deep Well Tip Comb in one MME-96 Standard Plate.
3. Add 150 µL of prepared Wash Solution 1 to two MME-96 Standard Plates.
4. Add 150 µL of prepared Wash Solution 2 to two MME-96 Standard Plates.
5. Add 90 µL of Elution Buffer to one MME-96 Standard Plate (not the tip comb plate).
6. Prepare the sample plate:

   IMPORTANT! 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 of 100% isopropanol to the plate.
7. Immediately start the MME-96 processor script, then load the plates onto the processor as directed.

Table 4 MME-96 processor protocol for semen samples

Sample volume		**115 µL of clarified lysate**		
MME-96 script		**4462359_DW_50**		
Machine configuration		**MME-96 Deep Well Magnetic Head**		
Tip comb		**MME-96 Deep Well Tip Comb**		
Plate position		**Reagent**	**Volume per well**	**Plate to use**
Plate	**Description**			
1	Sample plate	Bead Mix	20 µL	MME-96 Deep Well Plate
		Sample (clarified lysate)	115 µL	
		100% Isopropanol	65 µL	
2	First Wash 1	Wash Solution 1	150 µL	MME-96 Standard Plate
3	Second Wash 1	Wash Solution 1	150 µL	MME-96 Standard Plate
4	First Wash 2	Wash Solution 2	150 µL	MME-96 Standard Plate
5	Second Wash 2	Wash Solution 2	150 µL	MME-96 Standard Plate
6	Elution	Elution Buffer	90 µL	MME-96 Standard Plate
7	Tip comb plate	MME-96 Deep Well Tip Comb in plate		MME-96 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.

MME-24 processor

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 5 MME-24 processor protocol for semen samples

Sample volume		**115 µL of clarified lysate**	
MME-24 script		**4462359**	
Plate to use		**MME-24 Plate**	
Tip comb		**MME-24 Tip Comb**	
Well position		**Reagent**	**Volume per well**
Row	**Description**		
A	Sample wells	Bead Mix	20 µL
		Sample (clarified lysate)	115 µL
		100% Isopropanol	65 µL
B	First Wash 1	Wash Solution 1	150 µL
C	Second Wash 1	Wash Solution 1	150 µL
D	First Wash 2	Wash Solution 2	150 µL
E	Second Wash 2	Wash Solution 2	150 µL
F	Elution	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.

Section 4 Oral Fluid Samples

This section covers:

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" (this page) – Recommended for ≤24 samples
- "Prepare the lysate using plates" (this page) – Recommended for >24 samples

Prepare the lysate using microcentrifuge tubes

This method is recommended for ≤24 samples.

For each sample:

1. Using a repeater pipette, add 450 µL of the prepared Lysis/Binding Solution to a 1.5-mL microcentrifuge tube.
2. To ensure uniform sampling, vortex the oral fluid vigorously (maximum setting) for 15 seconds, then proceed immediately to step 3 below.
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 × *g* (maximum setting) for 2 minutes to clarify the lysate.

Proceed to "Purify the nucleic acid" on page 30.

Prepare the lysate using plates

This method is recommended for >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. To ensure uniform sampling, vortex the oral fluid vigorously (maximum setting) for 15 seconds, then proceed immediately to step 3 below.
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 page 13 for shaker settings).
6. Centrifuge at ≥2500 × *g* (maximum setting) for 5 minutes to clarify the lysate.

Proceed to "Purify the nucleic acid" (this page).

Purify the nucleic acid

This section provides procedures for purifying nucleic acid on the MagMAX™ Express-96 Deep Well Magnetic Particle Processor (MME-96 processor).

Note: For oral fluid samples, we determined that 300 µL is the optimal input volume. Because Deep Well Plates are required for ≥100-µL volumes, we have not included MagMAX™ Express Magnetic Particle Processor (MME-24 processor) or manual purification procedures.

MME-96 processor

IMPORTANT! 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 **4462359_DW_HV** MME-96 script.
2. Prepare the tip comb plate: Place an MME-96 Deep Well Tip Comb in one MME-96 Standard Plate.
3. Add 300 µL of prepared Wash Solution 1 to two MME-96 Deep Well Plates.
4. Add 450 µL of prepared Wash Solution 2 to two MME-96 Deep Well Plates.
5. Add 90 µL of Elution Buffer to one MME-96 Standard Plate (not the tip comb plate).
6. Prepare the sample plate:

   IMPORTANT! 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 6 MME-96 processor protocol for oral fluid samples

Sample volume		**600 µL of clarified lysate**		
MME-96 script		**4462359_DW_HV**		
Machine configuration		**MME-96 Deep Well Magnetic Head**		
Tip comb		**MME-96 Deep Well Tip Comb**		
Plate position		**Reagent**	**Volume per well**	**Plate to use**
Plate	**Description**			
1	Sample plate	Bead Mix	20 µL	MME-96 Deep Well Plate
		Sample (clarified lysate)	600 µL	
		100% Isopropanol	350 µL	
2	First Wash 1	Wash Solution 1	300 µL	MME-96 Deep Well Plate
3	Second Wash 1	Wash Solution 1	300 µL	MME-96 Deep Well Plate
4	First Wash 2	Wash Solution 2	450 µL	MME-96 Deep Well Plate
5	Second Wash 2	Wash Solution 2	450 µL	MME-96 Deep Well Plate
6	Elution	Elution Buffer	90 µL	MME-96 Standard Plate
7	Tip comb plate	MME-96 Deep Well Tip Comb in plate		MME-96 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.

Section 5 Fecal Samples

This section covers:

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

IMPORTANT! Do not centrifuge at higher speeds or centrifuge longer than 5 seconds. Otherwise, the bacteria may pellet.

Proceed to:

- "Prepare the lysate using microcentrifuge tubes" on page 34 – Recommended for ≤24 samples
- "Prepare the lysate using plates" on page 34 – Recommended for >24 samples

Prepare the lysate using microcentrifuge tubes

This method is recommended for ≤24 samples.

For each sample:

1. Using a repeater pipette, add 500 µL of the prepared Lysis/Binding Solution to a 1.5-mL microcentrifuge tube.
2. Using a large-bore pipette tip, 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.

   If particulates are visible: Centrifuge at 16,000 × *g* for 2 minutes.

Proceed to "Purify the nucleic acid" on page 35.

Prepare the lysate using plates

This method is recommended for >24 samples.

1. Using a multichannel pipette, add 500 µL of the prepared Lysis/Binding Solution to each reaction well of a MagMAX™ Express-96 Deep Well Plate.
2. Using a large-bore pipette tip, 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 page 13 for shaker settings).
5. Centrifuge at ≥2500 × *g* (maximum setting) for 5 minutes to clarify the lysate.

Proceed to "Purify the nucleic acid" on page 35.

Purify the nucleic acid

This section provides procedures for purifying nucleic acid on the 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 Appendix A on page 49.

MME-96 processor

IMPORTANT! 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 appropriate MME-96 script:
   - For 115 µL of clarified lysate, select **4462359_DW_50**
   - For 400 µL of clarified lysate, select **4462359_DW_HV**
2. Prepare the tip comb plate: Place an MME-96 Deep Well Tip Comb in one MME-96 Standard Plate.
3. Add prepared Wash Solution 1:
   - For 115 µL of clarified lysate, add 150 µL to two MME-96 Standard Plates.
   - For 400 µL of clarified lysate, add 300 µL to two MME-96 Deep Well Plates.
4. Add prepared Wash Solution 2:
   - For 115 µL of clarified lysate, add 150 µL to two MME-96 Standard Plates.
   - For 400 µL of clarified lysate, add 450 µL to two MME-96 Deep Well Plates.
5. Add 90 µL of Elution Buffer to one MME-96 Standard Plate (not the tip comb plate).
6. Prepare the sample plate:

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

   a. Add 20 µL of prepared Bead Mix to one MME-96 Deep Well Plate.
   b. Add prepared sample (clarified lysate) to the plate according to Table 7 on page 36.
   c. Add 100% isopropanol to the plate according to Table 7 on page 36.
7. Immediately start the MME-96 processor script, then load the plates onto the processor as directed.

Table 7 MME-96 processor protocol for fecal samples

Sample volume		**115 µL or 400 µL of clarified lysate**				
MME-96 script		**4462359_DW_50 (For 115-µL sample volumes)** **4462359_DW_HV (For 400-µL sample volumes)**				
Machine configuration		**MME-96 Deep Well Magnetic Head**				
Tip comb		**MME-96 Deep Well Tip Comb**				
Plate position		**Reagent**	**Volume per well**		MME-96 p**late to use for each sample volume**	
Plate	**Description**				**115 µL**	**400 µL**
1	Sample plate	Bead Mix	20 µL	20 µL	Deep Well	Deep Well
		Sample (clarified lysate)	115 µL	400 µL		
		100% Isopropanol	65 µL	350 µL		
2	First Wash 1	Wash Solution 1	150 µL	300 µL	Standard	Deep Well
3	Second Wash 1	Wash Solution 1	150 µL	300 µL	Standard	Deep Well
4	First Wash 2	Wash Solution 2	150 µL	450 µL	Standard	Deep Well
5	Second Wash 2	Wash Solution 2	150 µL	450 µL	Standard	Deep Well
6	Elution	Elution Buffer	90 µL	90 µL	Standard	Standard
7	Tip comb plate	MME-96 Deep Well Tip Comb in plate			Standard	Standard

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.

MME-24 processor

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 8 MME-24 processor protocol for fecal samples

Sample volume		**115 µL of clarified lysate**	
MME-24 script		**4462359**	
Plate to use		**MME-24 Plate**	
Tip comb		**MME-24 Tip Comb**	
Well position		**Reagent**	**Volume per well**
Row	**Description**		
A	Sample wells	Bead Mix	20 µL
		Sample (clarified lysate)	115 µL
		100% Isopropanol	65 µL
B	First Wash 1	Wash Solution 1	150 µL
C	Second Wash 1	Wash Solution 1	150 µL
D	First Wash 2	Wash Solution 2	150 µL
E	Second Wash 2	Wash Solution 2	150 µL
F	Elution	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.

Section 6 InPouch™ TF culture

This section covers:

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

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 page 40.

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.

   IMPORTANT! 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 × *g* (maximum setting) for 3 minutes.
5. Using a disposable pipette, carefully aspirate and discard all supernatant without disturbing the pellet.
6. Using p1000 pipette tips, add 1000 µL of sterile 1× PBS to the tube.
7. Vortex vigorously (maximum setting) for ~15 seconds, until the solution is uniformly suspended.

Proceed to:

- "Prepare the lysate using microcentrifuge tubes" on page 40 – Recommended for ≤24 samples
- "Prepare the lysate using plates" on page 40 – Recommended for >24 samples

Prepare the lysate using microcentrifuge tubes

This method is recommended for ≤24 samples.

For each sample:

1. Using a repeater pipette, add 150 µL of the prepared Lysis/Binding Solution to a 1.5-mL microcentrifuge tube.
2. To ensure uniform sampling, vortex the pellet suspension vigorously (maximum setting) for 15 seconds, then proceed immediately to step 3.
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 × *g* (maximum setting) for 2 minutes to clarify the lysate.

Proceed to "Purify the nucleic acid" on page 41. If you are using the MagMAX™ Express-96 Deep Well Magnetic Particle Processor, follow the MME-96 script for 115 µL of clarified lysate on page 41.

Prepare the lysate using plates

This method is recommended for >24 samples.

1. Using a multichannel pipette, add 150 µL of the prepared Lysis/Binding Solution to each reaction well of a MagMAX™ Express-96 Deep Well Plate.
2. To ensure uniform sampling, vortex the pellet suspension vigorously (maximum setting) for 15 seconds, then proceed immediately to step 3.
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 page 13 for shaker settings).
6. Centrifuge at ≥2500 × *g* (maximum setting) for 5 minutes to clarify the lysate.

Proceed to "Purify the nucleic acid" on page 41. If you are using the MagMAX™ Express-96 Deep Well Magnetic Particle Processor, follow the MME-96 script for 115 µL of clarified lysate on page 41.

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 page 39.

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.

   IMPORTANT! 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 page 42.

Purify the nucleic acid

This section provides procedures for purifying nucleic acid on the 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 Appendix A on page 49.

MME-96 processor

Clarified lysate

IMPORTANT! 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 **4462359_DW_50** MME-96 script.
2. Prepare the tip comb plate: Place an MME-96 Deep Well Tip Comb in one MME-96 Standard Plate.
3. Add 150 µL of prepared Wash Solution 1 to two MME-96 Standard Plates.
4. Add 150 µL of prepared Wash Solution 2 to two MME-96 Standard Plates.
5. Add 90 µL of Elution Buffer to one MME-96 Standard Plate (not the tip comb plate).
6. Prepare the sample plate:

   IMPORTANT! 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 9 MME-96 processor protocol for clarified InPouch™ TF culture lysate

Sample volume		**115 µL of clarified lysate**		
MME-96 script		**4462359_DW_50**		
Machine configuration		**MME-96 Deep Well Magnetic Head**		
Tip comb		**MME-96 Deep Well Tip Comb**		
Plate position		**Reagent**	**Volume per well**	**Plate to use**
Plate	**Description**			**115 µL**
1	Sample plate	Bead Mix	20 µL	MME-96 Deep Well Plate
		Sample (clarified lysate)	115 µL	
		100% Isopropanol	65 µL	
2	First Wash 1	Wash Solution 1	150 µL	MME-96 Standard Plate
3	Second Wash 1	Wash Solution 1	150 µL	MME-96 Standard Plate
4	First Wash 2	Wash Solution 2	150 µL	MME-96 Standard Plate
5	Second Wash 2	Wash Solution 2	150 µL	MME-96 Standard Plate
6	Elution	Elution Buffer	90 µL	MME-96 Standard Plate
7	Tip comb plate	MME-96 Deep Well Tip Comb in plate		MME-96 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.

InPouch™ TF culture

IMPORTANT! 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 **4462359_DW_HV** MME-96 script.
2. Prepare the tip comb plate: Place an MME-96 Deep Well Tip Comb in one MME-96 Standard Plate.
3. Add 300 µL of prepared Wash Solution 1 to two MME-96 Deep Well Plates.
4. Add 450 µL of prepared Wash Solution 2 to two MME-96 Deep Well Plates.
5. Add 90 µL of Elution Buffer to one MME-96 Standard Plate (not the tip comb plate).

6. Prepare the sample plate:

   IMPORTANT! 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 10 MME-96 processor protocol for InPouch™ TF culture

Sample volume		**300 µL of sample**		
MME-96 script		**4462359_DW_HV**		
Machine configuration		**MME-96 Deep Well Magnetic Head**		
Tip comb		**MME-96 Deep Well Tip Comb**		
Plate position		**Reagent**	**Volume per well**	**Plate to use**
Plate	**Description**			
1	Sample plate	Bead Mix	20 µL	MME-96 Deep Well Plate
		Sample	300 µL	
		Lysis/Binding Solution	700 µL	
2	First Wash 1	Wash Solution 1	300 µL	MME-96 Deep Well Plate
3	Second Wash 1	Wash Solution 1	300 µL	MME-96 Deep Well Plate
4	First Wash 2	Wash Solution 2	450 µL	MME-96 Deep Well Plate
5	Second Wash 2	Wash Solution 2	450 µL	MME-96 Deep Well Plate
6	Elution	Elution Buffer	90 µL	MME-96 Standard Plate
7	Tip comb plate	MME-96 Deep Well Tip Comb in plate		MME-96 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.

MME-24 processor – 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 11 MME-24 processor protocol for clarified InPouch™ TF culture lysate

Sample volume		**115 µL of clarified lysate**	
MME-24 script		**4462359**	
Plate to use		**MME-24 Plate**	
Tip comb		**MME-24 Tip Comb**	
Well position		**Reagent**	**Volume per well**
Row	**Description**		
A	Sample wells	Bead Mix	20 µL
		Sample (clarified lysate)	115 µL
		100% Isopropanol	65 µL
B	First Wash 1	Wash Solution 1	150 µL
C	Second Wash 1	Wash Solution 1	150 µL
D	First Wash 2	Wash Solution 2	150 µL
E	Second Wash 2	Wash Solution 2	150 µL
F	Elution	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.

Section 7 Analysis and Troubleshooting

This section covers:

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

Troubleshooting

Observation	Possible cause	Recommended action
Poor or no RNA or DNA signal (that is, the C_T 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.
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.
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" below 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.
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" above ("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.

Manual Purification Procedures

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 items are available from major laboratory suppliers (MLS).

IMPORTANT! For the SDS (Safety Data Sheet) of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

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 – various, including:					
• p1000	MLS	X	X	X	X
• Large-bore	MLS		X	X	
Note: Pipette tips should be aerosol-resistant and nuclease-free.					
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 (Part no. MPU-8117), or equivalent	X	X	X	X
• Untreated Lids for 96-Well Microplates, Clear	Phenix (Part no. MPE-8019), or equivalent	X	X	X	X
• MicroAmp® Clear Adhesive Film (for protecting unused wells of the 96-well processing plate)	Applied Biosystems (Part no. 4306311)	X	X	X	X
Magnetic stand, one of the following:					
• Magnetic Stand-96	Ambion (Part no. AM10027)	X	X	X	X
• 96 well Magnetic-Ring Stand	Ambion (Part no. AM10050)	X	X	X	X
Barnstead/Lab-Line Titer Plate Shaker	VWR (Part no. 57019-600), or equivalent	X	X	X	X
Hybridization oven or dry incubator	MLS	X	X	X	X

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*® surface decontamination solution	Ambion (Part no. AM9780)
DNA*Zap*™ surface decontamination spray	Ambion (Part no. 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	14
Wash Solution 2	14
Lysis/Binding Solution	15
Bead Mix	16

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 55).

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

Prepare the samples

Prepare your samples according to the appropriate procedure:

Sample type	See page...
Low-cell-content	17
Semen	25
Feces	33
InPouch™ TF culture	39

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 pages 25, 33, and 39).

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

Order to combine	Component	Sample type			
		Low-cell-content	Semen	Feces	InPouch™ TF culture
1	Prepared Bead Mix	20 µL	20 µL	20 µL	20 µL
2	Prepared sample	50 µL	115 µL	115 µL	115 µL
3	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 page 51 for shaker settings).
3. Use a magnetic stand to capture the beads. The capture time is ~1 to 3 minutes.
4. Carefully aspirate and discard all supernatant without disturbing the beads.
5. Remove the plate from the magnetic stand.

IMPORTANT! 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 page 51 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.

   IMPORTANT! It is critical that you remove the plate from the magnetic stand before the next step.
6. Repeat steps 1 to 5 above 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 page 51 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.

   IMPORTANT! It is critical that you remove the plate from the magnetic stand before the next step.
6. Repeat steps 1 to 5 above one time.

Dry the beads

1. Using a plate shaker, shake at vigorous speed for 2 minutes (see page 51 for shaker settings).
2. Inspect the plate. Aspirate and discard any residual Wash Solution 2.

Elute the nucleic acid

1. Add 90 µL of 65°C Elution Buffer (from page 52) to each reaction well.
2. Using a plate shaker, shake at vigorous speed for 3 minutes (see page 51 for shaker settings).

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

   If there are 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.

   IMPORTANT! Complete bead suspension is critical for high and consistent nucleic acid recovery.

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

Safety

This appendix covers:

Chemical safety

General chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- 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. (See "About SDSs" on page 58.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

SDSs

About SDSs

Chemical manufacturers supply current Safety Data Sheets (SDSs) with shipments of hazardous chemicals to new customers. They also provide SDSs with the first shipment of a hazardous chemical to a customer after an SDS has been updated. SDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new SDS packaged with a hazardous chemical, be sure to replace the appropriate SDS in your files.

Obtaining SDSs

The SDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain SDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **SDS**.

2. In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS of interest. Select the language of your choice, then click **Search**.

3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the SDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Safety Data Sheets and local regulations for handling and disposal.

WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide 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.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.

- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument 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.

Biological hazard safety

General biohazard

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. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* found at: **www.cdc.gov/biosafety/publications/index.htm**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/ nara/cfr/waisidx_01/ 29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

Documentation and Support

Product documentation

Portable document format (PDF) versions of the documents listed in this section are available at **www.appliedbiosystems.com**

Note: To open the PDF versions, use the Adobe Acrobat Reader software available from **www.adobe.com**

Document	Part number
MagMAX™ Pathogen RNA/DNA Kit Protocol	4463379
MagMAX™ Pathogen RNA/DNA Kit Quick Reference Card for Low-Cell-Content Samples	4463378

Obtaining support

For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

At the Applied Biosystems website, you can:

- Access worldwide telephone and fax numbers to contact Life Technologies Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, SDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

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Technical Resources and Support
For the latest technical resources and support information for all locations, please refer to our website at
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