MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit

High throughput isolation of Nucleic Acid (RNA and DNA) from fecal samples

Catalog Numbers A42357 (with plate), A42358 (with tubes)

Pub. No. MAN0018071 Rev. D

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Applied Biosystems™ MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit is developed for scalable, rapid purification of high-quality Total Nucleic Acid (RNA and DNA) from fecal samples. The nucleic acid purified with this kit can be used in a broad range of molecular biology downstream applications, such as sequencing and real-time PCR. This protocol guides users through automated isolation of RNA and DNA from fecal samples using the KingFisher™ Apex, KingFisher™ Flex, and KingFisher™ Duo Prime.

Contents and storage

Reagents that are provided in the kit are sufficient for 100 reactions (Cat. No. A42357 and A42358).

Table 1 MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit

Item	Amount	Storage
Lysis Buffer	80 mL	15°C to 25°C
Binding Solution	50 mL	
Wash Buffer	200 mL	
Elution Solution	20 mL	
Proteinase K	4 mL	
Total Nucleic Acid Binding Beads	2 mL	
Bead Tubes OR 96DW Bead Plate	100 tubes OR 1 plate	

For bulk reagents, use Cat. No. A42361 (Lysis Solution), A42359 (Binding Solution), A42360 (Wash Solution), A42364 (Elution Solution), A42363 (Proteinase K), and A42362 (Binding Beads).

For bead tubes and plate sold separately, use Cat. No. A42351 (Bead tubes, 100), and A42331 (Bead plate, 1).

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.

Item	Source
Magnetic particle processor (one of the following, depending on quantity/volume of sample to be processed):	
KingFisher™ Flex Purification System with 96 deep-well head	5400630
KingFisher™ Apex Purification System with 96 deep-well head	5400930
KingFisher™ Duo Prime Purification System	5400110
Equipment	
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Vortex with vortex adaptor capable of holding 2.0 mL bead-beating tubes	MLS
Bead-beating instrument for 96-well plates, such as Omni International Bead Ruptor 96 Well Plate Homogenizer	15-341-107 or equivalent
Microcentrifuge capable of reaching 14,000 x g, such as Sorvall™ Legend™ Micro 21R Microcentrifuge	75002435 or equivalent
Plate Centrifuge capable of reaching 2,250 × *g* and holding 96DW plates, such as Sorvall™ Legend™ XT/XF with M20 rotor	MLS

Item	Source
Consumables	
Deep-well plates:	
KingFisher™ Deepwell 96 Plate	95040450
96-well standard plates (for use with KingFisher™ Flex and KingFisher™ Apex only; tip comb placement and elution plate and/or eluate storage):	
KingFisher™ 96 KF microplate	97002540
Tip comb, compatible with the magnetic particle processor used:	
KingFisher™ Duo Prime 12-tip comb, for use with KingFisher™ deep-well 96 plate	97003500
KingFisher™ 96 tip comb for DW magnets, for Flex and Apex protocols only	97002534
Elution Strip, for Duo protocol only	97003520
KingFisher™ Duo Cap for Elution Strip, for Duo protocol only	97003540
Materials	
MicroAmp™ Clear Adhesive Film	4306311
Conical Tubes (15 mL)	AM12500
Conical Tubes (50 mL)	AM12501
Reagent reservoirs	MLS
Foil Seals	14-222-342
Nonstick, RNase-Free Microfuge Tubes, 1.5 mL	AM12450
Nonstick, RNase-Free Microfuge Tubes, 2.0 mL	AM12475
Reagents	
Ethanol, 100% (molecular biology grade)	MLS
Nuclease-free water	AM9932

General guidelines

- Perform all steps at room temperature (20–25°C), unless otherwise noted.
- Clean the work surfaces with RNA Zap to remove nucleases, then wipe the surfaces with 70% to 100% molecular biology grade ethanol to remove additional contaminants.
- Precipitates can occur if the Lysis Buffer or Binding Solution is stored when room temperature is too cold. If there are precipitates, warm the reagents at 37°C and gently mix to dissolve the precipitates. Avoid creating bubbles.

Guidelines for fecal samples

- Use freshly collected non-stabilized samples (within 2 hours of collection) or samples stored frozen in RNA-friendly fecal storage solution (such as RNALater).

Guidelines for Binding Bead Mix

- Vortex Binding Beads thoroughly before each use.
- Ensure that the beads stay fully mixed within the solution during pipetting.
- Avoid creating bubbles during mixing and aliquoting.
- Binding/Bead Mix is very viscous so pipet with care to ensure that the correct volume is added to the sample.

Before first use of the kit

Prepare 80% Ethanol from 100% absolute Ethanol and Nuclease-Free Water.

Prepare enough solution for a minimum volume of 2 mL per sample.

Before each use of the kit

1. Vortex Beads vigorously to ensure they are homogenous.
2. Prepare Binding/Bead Mix according to the following table:

Component	Volume per well[1]
Total Nucleic Acid Binding Buffer	500 µL
Total Nucleic Acid Magnetic Beads	20 µL
Total volume	**520 µL**

[1] Use 10% Overage calculation when making a master mix for use with multiple samples.

3. Mix well by inversion, then store at room temperature.

Perform nucleic acid purification using bead tubes and the KingFisher™ Flex instrument

1 Set up the instrument

1. Ensure that the instrument is set up with the proper magnetic head and the proper heat block, as indicated in the following table.

Component	Type
Magnetic head	96 deep-well magnetic head
Heat block	96 well deep-well heat block

IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields and potential harm to the instrument.

2. Ensure that the proper program (**MagMAX_Microbiome_Stool_Flex**) has been downloaded from the product page and loaded onto the instrument.

2 Lyse sample

1. Set up the vortex with the vortex adaptor.
2. Add 800 µL of Lysis Buffer to the bead tubes.
3. Prepare fecal samples according to the following table:

For	Description
Fresh or frozen fecal sample	Weigh out 100 mg, then place into prepared bead tube
Fecal sample in storage solution (stored at a 1:2 ratio fecal to solution)	Remove 200 µL, then place into prepared bead tube
Fecal swab	Remove the plastic stick, then place the swab into prepared bead tube

Note: For bacterial and yeast cultures, and urine, user can centrifuge 1 mL then re-suspend the cell pellet in 800 µL of Lysis Buffer and transfer to the bead tube to start the extraction.

4. Cap, then vortex the bead tube upside down for 10 seconds to mix the sample with the buffer.
5. Set the vortexer speed to 2,500 × *g*, then place the tubes onto the adaptor.

   Note: We recommend the VWR 24 tube adapter and vortex for maximum nucleic acid yield. If an alternative instrument or adaptor is used, ensure that the instrument can meet the speed listed in the protocol.

6. Lyse samples on the vortexer for 10 minutes.

STOPPING POINT Bead tube can be stored at 4°C overnight after lysis.

3 Set up the processing plates

Set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table.

Plate ID	Plate position	Plate type	Reagent	Volume per well
Wash 1 Plate	2	Deep-well	Wash Buffer	1,000 µL
Wash 2 Plate	3	Deep-well	Wash Buffer	1,000 µL
Wash 3 Plate	4	Deep-well	80% Ethanol	1,000 µL
Wash 4 Plate	5	Deep-well	80% Ethanol	1,000 µL
Elution Plate	6	Deep-well	Elution Solution	200 µL[1]
Tip Comb	7	Place a 96 Deep-well Tip Comb in a Standard deep-well plate		

[1] Elution volume can be reduced to 100 µL if using <50 mg of fecal sample or 1 fecal swab.

3 *(continued)*

Note: To prevent evaporation and contamination, cover the prepared processing plates with paraffin film or MicroAmp™ Clear Adhesive Film until they are loaded into the instrument.

4 Bind, wash, then elute total nucleic acid

1. Remove tubes from vortexer, then centrifuge for at 14,000 × *g* for 2 minutes.
2. Transfer up to 400 µL of the Sample to the appropriate wells of a new deep-well plate.

   This plate is the Sample Plate.

   STOPPING POINT Lysate can be stored at –20°C for up to 3 months. Before use, ensure sample has equilibrated to room temperature.
3. Invert Binding Bead Mix to mix, then add 520 µL to each sample in the Sample Plate.

   Note: Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. The mixture containing the Binding Beads is viscous. Therefore, pipet slowly to ensure that the correct amount is added. DO NOT use a repeat pipet to add to the samples as the high viscosity will cause variations in volume added.
4. Select the program **MagMAX_ Microbiome_Stool_Flex** on the instrument.
5. Start the run, then load the prepared sample and processing plates into position when prompted by the instrument.
6. After the protocol is complete (~35 minutes after start), immediately remove the elution plate from the instrument and cover the plate or transfer the eluate to the final tube or plate of choice for final storage.

The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for up to 6 months and –80°C for greater than 6 months.

Perform nucleic acid purification using bead tubes and the KingFisher™ Apex instrument

1 Set up the instrument

1. Ensure that the instrument is set up with the proper magnetic head and the proper heat block, as indicated in the following table.

Component	Type
Magnetic head	96 deep-well magnetic head
Heat block	96 well deep-well heat block

IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields and potential harm to the instrument.

2. Ensure that the proper program (**MagMAX_Microbiome_Stool**) has been downloaded from the product page and loaded onto the instrument.

2 Lyse sample

1. Set up the vortex with the vortex adaptor.
2. Add 800 µL of Lysis Buffer to the bead tubes.

2 *(continued)*

3. Prepare fecal samples according to the following table:

For	Description
Fresh or frozen fecal sample	Weigh out 100 mg, then place into prepared bead tube
Fecal sample in storage solution (stored at a 1:2 ratio fecal to solution)	Remove 200 µL, then place into prepared bead tube
Fecal swab	Remove the plastic stick, then place the swab into prepared bead tube

Note: For bacterial and yeast cultures, and urine, user can centrifuge 1 mL then re-suspend the cell pellet in 800 µL of Lysis Buffer and transfer to the bead tube to start the extraction.

4. Cap, then vortex the bead tube upside down for 10 seconds to mix the sample with the buffer.

5. Set the vortexer speed to 2,500 × *g*, then place the tubes onto the adaptor.

   Note: We recommend the VWR 24 tube adapter and vortex for maximum nucleic acid yield. If an alternative instrument or adaptor is used, ensure that the instrument can meet the speed listed in the protocol.

6. Lyse samples on the vortexer for 10 minutes.

STOPPING POINT Bead tube can be stored at 4°C overnight after lysis.

3 Set up the processing plates

Set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table.

Plate ID	Plate position	Plate type	Reagent	Volume per well
Wash 1 Plate	3	Deep-well	Wash Buffer	1,000 µL
Wash 2 Plate	4	Deep-well	Wash Buffer	1,000 µL
Wash 3 Plate	5	Deep-well	80% Ethanol	1,000 µL
Wash 4 Plate	6	Deep-well	80% Ethanol	1,000 µL
Elution Plate	7	Deep-well	Elution Solution	200 µL[1]
Tip Comb	1	Place a 96 Deep-well Tip Comb in a Standard deep-well Plate		

[1] Elution volume can be reduced to 100 µL if using <50 mg of fecal sample or 1 fecal swab.

Note: To prevent evaporation and contamination, cover the prepared processing plates with paraffin film or MicroAmp™ Clear Adhesive Film until they are loaded into the instrument.

4 Bind, wash, then elute total nucleic acid

1. Remove tubes from vortexer, then centrifuge at 14,000 × *g* for 2 minutes.

2. Transfer up to 400 µL of the sample to the appropriate wells of a new deep-well plate.

   This plate is the Sample Plate.

STOPPING POINT Lysate can be stored at –20°C for up to 3 months. Before use, ensure sample has equilibrated to room temperature.

3. Invert Binding Bead Mix to mix, then add 520 µL to each sample in the Sample Plate.

   Note: Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. The mixture containing the Binding Beads is viscous. Therefore, pipet slowly to ensure that the correct amount is added. DO NOT use a repeat pipet to add to the samples as the high viscosity will cause variations in volume added.

4. Select the program **MagMAX_ Microbiome_Stool** on the instrument.

4 *(continued)*

5. Start the run, then load the prepared sample and processing plates into position when prompted by the instrument.

6. After the protocol is complete (~35 minutes after start), immediately remove the elution plate from the instrument and cover the plate or transfer the eluate to the final tube or plate of choice for final storage.

The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for up to 6 months and –80°C for greater than 6 months.

Perform nucleic acid purification using bead plates and the KingFisher™ Flex instrument

1 Set up the instrument

1. Ensure that the instrument is set up with the proper magnetic head and the proper heat block, as indicated in the following table.

Component	Type
Magnetic head	96 deep-well magnetic head
Heat block	96 well deep-well heat block

IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields and potential harm to the instrument.

2. Ensure that the proper program (**MagMAX_Microbiome_Stool_Flex**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plates

Set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table.

Plate ID	Plate position	Plate type	Reagent	Volume per well
Wash 1 Plate	2	Deep-well	Wash Buffer	1,000 µL
Wash 2 Plate	3	Deep-well	Wash Buffer	1,000 µL
Wash 3 Plate	4	Deep-well	80% Ethanol	1,000 µL
Wash 4 Plate	5	Deep-well	80% Ethanol	1,000 µL
Elution Plate	6	Deep-well	Elution Solution	200 µL[1]
Tip Comb	7	Place a 96 Deep-well Tip Comb in a Standard deep-well plate		

[1] Elution volume can be reduced to 100 µL if using <50 mg of fecal sample or 1 fecal swab.

Note: To prevent evaporation and contamination, cover the prepared processing plates with paraffin film or MicroAmp™ Clear Adhesive Film until they are loaded into the instrument.

3 Lyse sample

1. Centrifuge the 96-well bead plate at 3,700–4,000 × *g* for 10 seconds to collect the beads on the bottom of the plate before opening the seal.

2. Open the seal of the bead plate, then add 800 µL of Lysis Buffer to each well.

3 *(continued)*

3. Prepare fecal samples according to the following table:

For	Description
Fresh or frozen fecal sample	Weigh out 100 mg, then place into prepared well in bead plate
Fecal sample in storage solution (stored at a 1:2 ratio fecal to solution)	Remove 200 µL, then place into prepared well in bead plate
Fecal swab	Remove the plastic stick, then place the swab into prepared well in bead plate

Note: Use a lint-free cloth to wipe down the top of the wells and edges to remove any sample, bead material, and ensure proper sealing and prevent leaks.

4. Seal the bead plate with MicroAmp™ Clear Adhesive Film by pressing down around each well and the edges of the plate using your thumb or adhesive film applicator.

   Note: For detailed instructions on plate sealing, see *MagMAX™ Bead beating plate guidelines Quick Reference* (MAN0018558).

5. Repeat step 3.4 twice with foil seals.

   Note: Ensure the edges and all the wells of the plate are sealed properly.

6. Set the bead beater for 2 minutes, then clamp the plate in place.
   - Omni Bead Ruptor 96: Set at 30 Hz for 2 minutes
   - Mini Bead Beater 96: Set for 2 minutes
   - Vortex with plate adaptor: Set at 2,000 × *g* for 5 minutes

7. Lyse the samples on the bead beater for 2 minutes.

STOPPING POINT Bead plate can be stored at 4°C overnight after lysis.

4 Bind, wash, then elute total nucleic acid

1. Remove the bead plate from the instrument, then centrifuge at 3,700 × *g* for 5 minutes.

   Note: Do not remove the plate seal until the plate has been centrifuged after the bead beating.

2. Transfer up to 400 µL of the sample to the appropriate wells of a new deep-well plate.

   This plate is the Sample Plate.

STOPPING POINT Lysate can be stored at –20°C for up to 3 months. Before use, ensure sample has equilibrated to room temperature.

3. Invert Binding Bead Mix to mix, then add 520 µL to each sample in the Sample Plate.

   Note: Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. The mixture containing the Binding Beads is viscous. Therefore, pipet slowly to ensure that the correct amount is added. DO NOT use a repeat pipet to add to the samples as the high viscosity will cause variations in volume added.

4. Select the program **MagMAX_ Microbiome_Stool_Flex** on the instrument.

5. Start the run, then load the prepared plates into position when prompted by the instrument.

6. After the protocol is complete (~35 minutes after start), immediately remove the elution plate from the instrument and cover the plate or transfer the eluate to the final tube or plate of choice for final storage.

The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for up to 6 months and –80°C for greater than 6 months.

Perform nucleic acid purification using bead plates and the KingFisher™ Apex instrument

1 Set up the instrument

1. Ensure that the instrument is set up with the proper magnetic head and the proper heat block, as indicated in the following table.

Component	Type
Magnetic head	96 deep-well magnetic head
Heat block	96 well deep-well heat block

IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields and potential harm to the instrument.

2. Ensure that the proper program (**MagMAX_Microbiome_Stool**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plates

Set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table.

Plate ID	Plate position	Plate type	Reagent	Volume per well
Wash 1 Plate	3	Deep-well	Wash Buffer	1,000 µL
Wash 2 Plate	4	Deep-well	Wash Buffer	1,000 µL
Wash 3 Plate	5	Deep-well	80% Ethanol	1,000 µL
Wash 4 Plate	6	Deep-well	80% Ethanol	1,000 µL
Elution Plate	7	Deep-well	Elution Solution	200 µL[1]
Tip Comb	1	Place a 96 Deep-well Tip Comb in a Standard deep-well Plate		

[1] Elution volume can be reduced to 100 µL if using <50 mg of fecal sample or 1 fecal swab.

Note: To prevent evaporation and contamination, cover the prepared processing plates with paraffin film or MicroAmp™ Clear Adhesive Film until they are loaded into the instrument.

3 Lyse sample

1. Centrifuge the 96-well bead plate at 3,700–4,000 × *g* for 10 seconds to collect the beads on the bottom of the plate before opening the seal.

2. Open the seal of the bead plate, then add 800 µL of Lysis Buffer to each well.

3. Prepare fecal samples according to the following table:

For	Description
Fresh or frozen fecal sample	Weigh out 100 mg, then place into prepared well in bead plate
Fecal sample in storage solution (stored at a 1:2 ratio fecal to solution)	Remove 200 µL, then place into prepared well in bead plate
Fecal swab	Remove the plastic stick, then place the swab into prepared well in bead plate

Note: Use a lint-free cloth to wipe down the top of the wells and edges to remove any sample, bead material, and ensure proper sealing and prevent leaks.

4. Seal the bead plate with MicroAmp™ Clear Adhesive Film by pressing down around each well and the edges of the plate using your thumb or adhesive film applicator.

   Note: For detailed instructions on plate sealing, see *MagMAX™ Bead beating plate guidelines Quick Reference* (MAN0018558).

5. Repeat step 3.4 twice with foil seals.

   Note: Ensure the edges and all the wells of the plate are sealed properly.

3 *(continued)*

6. Set the bead beater for 2 minutes, then clamp the plate in place.
   - Omni Bead Ruptor 96: Set at 30 Hz for 2 minutes
   - Mini Bead Beater 96: Set for 2 minutes
   - Vortex with plate adaptor: Set at 2,000 × *g* for 5 minutes
7. Lyse the samples on the bead beater for 2 minutes.

STOPPING POINT Bead plate can be stored at 4°C overnight after lysis.

4 Bind, wash, then elute total nucleic acid

1. Remove the bead plate from the instrument, then centrifuge at 3,700 × *g* for 5 minutes.

   Note: Do not remove the plate seal until the plate has been centrifuged after the bead beating.
2. Transfer up to 400 µL of the sample to the appropriate wells of a new deep-well plate.

   This plate is the Sample Plate.

   STOPPING POINT Lysate can be stored at –20°C for up to 3 months. Before use, ensure sample has equilibrated to room temperature.
3. Invert Binding Bead Mix to mix, then add 520 µL to each sample in the Sample Plate.

   Note: Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. The mixture containing the Binding Beads is viscous. Therefore, pipet slowly to ensure that the correct amount is added. DO NOT use a repeat pipet to add to the samples as the high viscosity will cause variations in volume added.
4. Select the program **MagMAX_ Microbiome_Stool** on the instrument.
5. Start the run, then load the prepared plates into position when prompted by the instrument.
6. After the protocol is complete (~35 minutes after start), immediately remove the elution plate from the instrument and cover the plate or transfer the eluate to the final tube or plate of choice for final storage.

The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for up to 6 months and –80°C for greater than 6 months.

Perform nucleic acid purification using bead tubes and the KingFisher™ Duo Prime instrument

1 Set up the instrument

1. Ensure that the instrument is set up with the proper magnetic head and the proper heat block, as indicated in the following table.

Component	Type
Magnetic head	12-tip magnetic head
Heat block	12 well deep-well heat block

IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields and potential harm to the instrument.

2. Ensure that the proper program (**MagMAX_Microbiome_Stool_Duo**) has been downloaded from the product page and loaded onto the instrument.

2 Lyse sample

1. Set up the vortex with the vortex adaptor.
2. Add 800 µL of Lysis Buffer to the bead tubes.
3. Prepare fecal samples according to the following table:

For	Description
Fresh or frozen fecal sample	Weigh out 100 mg, then place into prepared bead tube
Fecal sample in storage solution (stored at a 1:2 ratio fecal to solution)	Remove 200 µL, then place into prepared bead tube
Fecal swab	Remove the plastic stick, then place the swab into prepared bead tube

Note: For bacterial and yeast cultures, and urine, user can centrifuge 1 mL then re-suspend the cell pellet in 800 µL of Lysis Buffer and transfer to the bead tube to start the extraction.

4. Cap, then vortex the bead tube upside down for 10 seconds to mix the sample with the buffer.
5. Set the vortexer speed to 2,500 × *g*, then place the tubes onto the adaptor.

   Note: We recommend the VWR 24 tube adapter and vortex for maximum nucleic acid yield. If an alternative instrument or adaptor is used, ensure that the instrument can meet the speed listed in the protocol.

6. Lyse samples on the vortexer for 10 minutes.

STOPPING POINT Bead tube can be stored at 4°C overnight after lysis.

3 Set up the Sample Plate

Set up the Sample Plate according to the following table.

Row ID	Plate Row	Reagent	Volume per well
Elution Solution	A	Elution Solution	200 µL[1]
Tip Comb	B	Tip Comb	Empty
—	C	Empty	
80% Ethanol	D	80% Ethanol	1,000 µL
80% Ethanol	E	80% Ethanol	1,000 µL
Wash Buffer	F	Wash Buffer	1,000 µL
Wash Buffer	G	Wash Buffer	1,000 µL
Sample row	H	Sample	Varies

[1] Elution volume can be reduced to 100 µL if using <50 mg of fecal sample or 1 fecal swab.

Note: To prevent evaporation and contamination, cover the prepared processing plates with paraffin film or MicroAmp™ Clear Adhesive Film until they are loaded into the instrument.

4 Bind, wash, then elute total nucleic acid

1. Remove tubes from vortexer, then centrifuge at 14,000 × *g* for 2 minutes.
2. Transfer up to 400 µL of the sample to the appropriate wells in Row H of the Sample Plate.

   This plate is the Sample Plate.

   STOPPING POINT Lysate can be stored at –20°C for up to 3 months. Before use, ensure sample has equilibrated to room temperature.

3. Invert Binding Bead Mix to mix, then add 520 µL to each sample in Row H of the Sample Plate.

   Note: Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. The mixture containing the Binding Beads is viscous. Therefore, pipet slowly to ensure that the correct amount is added. DO NOT use a repeat pipet to add to the samples as the high viscosity will cause variations in volume added.

4. Select the program **MagMAX_ Microbiome_Stool_Duo** on the instrument.
5. Start the run, then load the prepared plate into position when prompted by the instrument.
6. After the protocol is complete (~35 minutes after start), immediately remove the plate from the instrument and transfer the eluate to the final tube or plate of choice for final storage.

The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for up to 6 months and –80°C for greater than 6 months.

Perform nucleic acid purification using bead plates and the KingFisher™ Duo Prime instrument

1 Set up the instrument

1. Ensure that the instrument is set up with the proper magnetic head and the proper heat block, as indicated in the following table.

Component	Type
Magnetic head	12-tip magnetic head
Heat block	12 well deep-well heat block

IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields and potential harm to the instrument.

2. Ensure that the proper program (**MagMAX_Microbiome_Stool_Duo**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the Sample Plate

Set up the Sample Plate according to the following table.

Row ID	Plate Row	Reagent	Volume per well
Elution Solution	A	Elution Solution	200 µL[1]
Tip Comb	B	Tip Comb	Empty
—	C	Empty	
80% Ethanol	D	80% Ethanol	1,000 µL
80% Ethanol	E	80% Ethanol	1,000 µL
Wash Buffer	F	Wash Buffer	1,000 µL
Wash Buffer	G	Wash Buffer	1,000 µL
Sample row	H	Sample	Varies

[1] Elution volume can be reduced to 100 µL if using <50 mg of fecal sample or 1 fecal swab.

Note: To prevent evaporation and contamination, cover the prepared processing plates with paraffin film or MicroAmp™ Clear Adhesive Film until they are loaded into the instrument.

3 Lyse sample

1. Centrifuge the 96-well bead plate at 3,700–4,000 × *g* for 10 seconds to collect the beads on the bottom of the plate before opening the seal.
2. Open the seal of the bead plate, then add 800 µL of Lysis Buffer to each well.
3. Prepare fecal samples according to the following table:

For	Description
Fresh or frozen fecal sample	Weigh out 100 mg, then place into prepared well in bead plate
Fecal sample in storage solution (stored at a 1:2 ratio fecal to solution)	Remove 200 µL, then place into prepared well in bead plate
Fecal swab	Remove the plastic stick, then place the swab into prepared well in bead plate

Note: Use a lint-free cloth to wipe down the top of the wells and edges to remove any sample, bead material, and ensure proper sealing and prevent leaks.

4. Seal the bead plate with MicroAmp™ Clear Adhesive Film by pressing down around each well and the edges of the plate using your thumb or adhesive film applicator.

   Note: For detailed instructions on plate sealing, see *MagMAX™ Bead beating plate guidelines Quick Reference* (MAN0018558).

3 *(continued)*

5. Repeat step 3.4 twice with foil seals.

   Note: Ensure the edges and all the wells of the plate are sealed properly.

6. Set the bead beater for 2 minutes, then clamp the plate in place.
   - Omni Bead Ruptor 96: Set at 30 Hz for 2 minutes
   - Mini Bead Beater 96: Set for 2 minutes
   - Vortex with plate adaptor: Set at 2,000 × *g* for 5 minutes

7. Lyse the samples on the bead beater for 2 minutes.

STOPPING POINT Bead plate can be stored at 4°C overnight after lysis.

4 **Bind, wash, then elute total nucleic acid**

1. Remove the bead plate from the instrument, then centrifuge at 3,700 × *g* for 5 minutes.

   Note: Do not remove the plate seal until the plate has been centrifuged after the bead beating.

2. Transfer up to 400 µL of the sample to the appropriate wells of Row H in the Sample Plate.

STOPPING POINT Lysate can be stored at –20°C for up to 3 months. Before use, ensure sample has equilibrated to room temperature.

3. Invert Binding Bead Mix to mix, then add 520 µL to each sample in the Sample Plate.

   Note: Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. The mixture containing the Binding Beads is viscous. Therefore, pipet slowly to ensure that the correct amount is added. DO NOT use a repeat pipet to add to the samples as the high viscosity will cause variations in volume added.

4. Select the program **MagMAX_ Microbiome_Stool_Duo** on the instrument.

5. Start the run, then load the prepared plates into position when prompted by the instrument.

6. After the protocol is complete (~35 minutes after start), immediately remove the plate from the instrument and transfer the eluate to the final tube or plate of choice for final storage.

The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for up to 6 months and –80°C for greater than 6 months.

Limited product warranty

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

Life Technologies Corporation | 2130 Woodward Street | Austin, TX 78744

Revision history: Pub. No. MAN0018071 D

Revision	Date	Description
D	10 October 2024	The document was updated to include support and consumables for the KingFisher™ Apex Purification System.
C.0	27 July 2019	In contents and storage, changed For 1,000 reaction volume to For bulk reagents.
B.0	22 April 2019	Updated sku for bead plate in Contents and storage from A423331 to A42331.
A.0	17 March 2019	New document.