MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit

Manual isolation of Nucleic Acid (RNA and DNA) from fecal, soil, biofluids, and other samples

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

Pub. No. MAN0018069 **Rev.** C.0

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, soil, biofluids, and other samples. You can use the nucleic acid purified with this kit in a broad range of molecular biology downstream applications, such as sequencing and real-time PCR. This protocol guides users through manual isolations using a bead tube or plate format.

Contents and storage

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

Table 1 Components of MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit

Component	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: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
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 (e.g. OMNI bead-ruptor)	MLS
Tube Centrifuge capable of reaching 14,000 × *g* and holding 2 mL tubes (e.g. Sorvall Legend Micro 21 Centrifuge, Ventilated; Thermofisher.com 75002435)	MLS
Plate Centrifuge capable of reaching 2,250 × *g* and holding 96DW plates (e.g. Thermo Scientific™ Sorvall™ Legend™ XT/XF with M20 rotor)	MLS
Magnetic Stand-96	AM10027
Compact Digital Microplate Shaker	88880023
Incubator capable of reaching 75°C with slatted shelves	MLS
Consumables	
Deep-well plates:	
KingFisher™ Deepwell 96 Plate	95040450
KingFisher™ 96 KF microplate	97002540
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

Item	Source
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.
- If using a plate shaker other than the recommended shaker, ensure that:
  - The plate fits securely on the plate shaker.
  - The recommended speeds are compatible with the plate shaker. Ideal shaker speeds allow for thorough mixing without splashing.
- *For soil samples only*: In rare instances, if some inhibitors still remain within the purified DNA/RNA, the sample can be diluted 20x with nuclease-free water prior to downstream PCR or other reactions.

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 from fecal samples using Bead tubes

1 Lyse sample

a. Set up the Vortex with the vortex adaptor.

b. Add 800 µL of Lysis Buffer to the bead tubes.

c. 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

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

e. Set the vortexer speed to 2,500 rpm, 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.

1 Lyse sample *(continued)*

f. Lyse samples on the vortexer for 10 minutes.

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

2 Bind the beads

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

b. 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.

c. 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.

d. Seal the plate with MicroAmp™ Clear Adhesive Film, ensuring that it is adequately sealed around the individual wells.

e. Shake the sealed plate at 900 rpm for 5 minutes.

f. Place the sealed plate on the magnetic stand for at least 5 minutes, or until all the beads have collected.

3 Wash the beads

a. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

IMPORTANT! Avoid disturbing the beads.

b. Remove the plate from the magnetic stand, then add 1 mL of Wash Buffer to each sample.

c. Reseal the plate, then shake at 800 rpm for 30 seconds.

d. Place the plate back on the magnetic stand for 3 minutes, or until all the beads have collected.

e. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

IMPORTANT! Avoid disturbing the beads.

f. Repeat step 3b to step 3e using 1 mL of Wash Buffer.

g. Repeat step 3b to step 3e using 1 mL of 80% Ethanol.

h. Repeat step 3b to step 3e using 1 mL of 80% Ethanol.

i. Dry the beads by shaking the plate (uncovered) at 800 rpm for 2 minutes.

4 Elute the nucleic acid

a. Add 200 µL of Elution Solution to each sample, then seal the plate with MicroAmp™ Clear Adhesive Film.

b. Place the plate in an incubator at 75°C for 5 minutes.

c. Remove the plate from the incubator, then place on a shaker at 800 rpm for 5 minutes.

d. Place the sealed plate on the magnetic stand for 3 minutes or until all beads are collected against the magnets.

4 Elute the nucleic acid *(continued)*

e. Keep the plate on the magnet and carefully remove the seal, then transfer the eluates to a fresh standard (not deep-well) plate.

IMPORTANT! To prevent evaporation, seal the plate containing the eluate immediately after the transfers are complete.

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 from fecal samples using Bead plates

1 Lyse sample

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

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

c. 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 Kim wipe to wipe down the top of the wells and edges to remove any sample or bead material and ensure proper sealing and prevent leaks.

d. 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).

e. Repeat step 1d twice with foil seals.

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

f. 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 rpm for 5 minutes

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

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

2 Bind the beads

a. Remove the bead plate from the instrument, then centrifuge for 5 minutes at 3,700 rpm.

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

b. Transfer 400–500 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.

2 Bind the beads *(continued)*

c. 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.

d. Seal the plate with MicroAmp™ Clear Adhesive Film, ensuring that it is adequately sealed around the individual wells.

e. Shake the sealed plate at 900 rpm for 5 minutes.

f. Place the sealed plate on the magnetic stand for at least 5 minutes, or until all the beads have collected.

3 Wash the beads

a. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

   IMPORTANT! Avoid disturbing the beads.

b. Remove the plate from the magnetic stand, then add 1 mL of Wash Buffer to each sample.

c. Reseal the plate, then shake at 800 rpm for 30 seconds.

d. Place the plate back on the magnetic stand for 3 minutes, or until all the beads have collected.

e. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

   IMPORTANT! Avoid disturbing the beads.

f. Repeat step 3b to step 3e using 1 mL of Wash Buffer.

g. Repeat step 3b to step 3e using 1 mL of 80% Ethanol.

h. Repeat step 3b to step 3e using 1 mL of 80% Ethanol.

i. Dry the beads by shaking the plate (uncovered) at 800 rpm for 2 minutes.

4 Elute the nucleic acid

a. Add 200 µL of Elution Solution to each sample, then seal the plate with MicroAmp™ Clear Adhesive Film.

b. Place the plate in an incubator at 75°C for 5 minutes.

c. Remove the plate from the incubator, then place on a shaker at 800 rpm for 5 minutes.

d. Place the sealed plate on the magnetic stand for 3 minutes or until all beads are collected against the magnets.

e. Keep the plate on the magnet and carefully remove the seal, then transfer the eluates to a fresh standard (not deep-well) plate.

   IMPORTANT! To prevent evaporation, seal the plate containing the eluate immediately after the transfers are complete.

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 total nucleic acid purification from soil, biofluids, and other samples using Bead tubes

1 Lyse sample

a. Set up the Vortex with the vortex adaptor.

b. Add 800 µL of Lysis Buffer to the bead tubes.

c. Prepare samples according to the following table:

Sample type	Description
Soil Samples	Weigh out 200–250 mg, then place into prepared bead tube
Biofluid or Liquid sample	Remove 400–500 µL, then place into prepared bead tube
Skin/Buccal Swab	Remove the plastic stick, then place the swab into prepared bead tube
Bacterial/Yeast Culture	Remove 500 µL of the culture, then place 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.

d. Vortex the bead tube upside down for 10 seconds to mix the sample with the buffer.

e. Set the speed to 2,500 rpm, then place the tubes onto the vortex 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.

f. Lyse samples on the vortexer for 10 minutes.

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

2 Digest with Proteinase K

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

b. Transfer 400–500 µL of the Sample to the appropriate wells of a new deep-well plate.

This plate is the Sample Plate.

Note: For soil samples, transfer as much supernatant as possible without any particle carryover.

c. Add 40 µL of Proteinase K to each sample.

d. Seal the plate with MicroAmp™ Clear Adhesive Film, ensuring that it is adequately sealed around the individual wells.

e. Shake the sealed plate at 900 rpm for 5 minutes.

f. Place the plate in an incubator at 65°C for 20 minutes.

Note: Ensure that there is free air flow to the bottom of the plate for proper sample heating.

3 Bind the beads

a. Remove plate from incubator

b. 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.

Note: Centrifuge the plate at 800 rpm for 1 min to collect liquid to bottom of plate if needed.

c. Seal the plate with MicroAmp™ Clear Adhesive Film, ensuring that it is adequately sealed around the individual wells.

3 Bind the beads *(continued)*

d. Shake the sealed plate at 900 rpm for 5 minutes.

e. Place the sealed plate on the magnetic stand for at least 5 minutes, or until all the beads have collected.

4 Wash the beads

a. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

IMPORTANT! Avoid disturbing the beads.

b. Remove the plate from the magnetic stand, then add 1 mL of Wash Buffer to each sample.

c. Reseal the plate, then shake at 800 rpm for 30 seconds.

d. Place the plate back on the magnetic stand for 3 minutes, or until all the beads have collected.

e. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

IMPORTANT! Avoid disturbing the beads.

f. Repeat step 4b to step 4e using 1 mL of Wash Buffer.

g. Repeat step 4b to step 4e using 1 mL of 80% Ethanol.

h. Repeat step 4b to step 4e using 1 mL of 80% Ethanol.

i. Dry the beads by shaking the plate (uncovered) at 800 rpm for 2 minutes.

5 Elute the nucleic acid

a. Add 50 µL of Elution Solution to each sample, then seal the plate with MicroAmp™ Clear Adhesive Film.

b. Place the plate in an incubator at 75°C for 5 minutes.

c. Remove the plate from the incubator, then place on a shaker at 800 rpm for 5 minutes.

d. Place the sealed plate on the magnetic stand for 3 minutes or until all beads are collected against the magnets.

e. Keep the plate on the magnet and carefully remove the seal, then transfer the eluates to a fresh standard (not deep-well) plate.

IMPORTANT! To prevent evaporation, seal the plate containing the eluate immediately after the transfers are complete.

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 from soil, biofluid, or other samples using Bead plates

1 Lyse sample

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

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

1 Lyse sample *(continued)*

c. Prepare samples according to the following table:

Sample type	Description
Soil Samples	Weigh out 200–250 mg, then place into prepared bead tube
Biofluid or Liquid sample	Remove 400–500 µL, then place into prepared bead tube
Skin/Buccal Swab	Remove the plastic stick, then place the swab into prepared bead tube
Bacterial/Yeast Culture	Remove 500 µL of the culture, then place 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.

Note: Use a Kim wipe to wipe down the top of the wells and edges to remove any sample or bead material and ensure proper sealing and prevent leaks.

d. 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).

e. Repeat step 1d twice with foil seals.

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

f. Set the bead beater for 2 minutes, then clamp the plate into 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 rpm for 5 minutes

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

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

2 Digest with Proteinase K

a. Remove the bead plate from the instrument, then centrifuge for 5 minutes at 3,700 rpm.

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

b. Transfer 400–500 µL of the Sample to the appropriate wells of a new deep-well plate.

This plate is the Sample Plate.

Note: For soil samples, transfer as much supernatant as possible without any particle carryover.

c. Add 40 µL of Proteinase K to each sample.

d. Seal the plate with MicroAmp™ Clear Adhesive Film, ensuring that it is adequately sealed around the individual wells.

e. Shake the sealed plate at 900 rpm for 5 minutes.

f. Place the plate in an incubator at 65°C for 20 minutes.

Note: Ensure that there is free air flow to the bottom of the plate for proper sample heating.

3 Bind the beads

a. Remove plate from incubator

b. 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.

3 Bind the beads *(continued)*

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.

Note: Centrifuge the plate at 800 rpm for 1 min to collect liquid to bottom of plate if needed.

c. Seal the plate with MicroAmp™ Clear Adhesive Film, ensuring that it is adequately sealed around the individual wells.

d. Shake the sealed plate at 900 rpm for 5 minutes.

e. Place the sealed plate on the magnetic stand for at least 5 minutes, or until all the beads have collected.

4 Wash the beads

a. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

IMPORTANT! Avoid disturbing the beads.

b. Remove the plate from the magnetic stand, then add 1 mL of Wash Buffer to each sample.

c. Reseal the plate, then shake at 800 rpm for 30 seconds.

d. Place the plate back on the magnetic stand for 3 minutes, or until all the beads have collected.

e. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

IMPORTANT! Avoid disturbing the beads.

f. Repeat step 4b to step 4e using 1 mL of Wash Buffer.

g. Repeat step 4b to step 4e using 1 mL of 80% Ethanol.

h. Repeat step 4b to step 4e using 1 mL of 80% Ethanol.

i. Dry the beads by shaking the plate (uncovered) at 800 rpm for 2 minutes.

5 Elute the nucleic acid

a. Add 50 µL of Elution Solution to each sample, then seal the plate with MicroAmp™ Clear Adhesive Film.

b. Place the plate in an incubator at 75°C for 5 minutes.

c. Remove the plate from the incubator, then place on a shaker at 800 rpm for 5 minutes.

d. Place the sealed plate on the magnetic stand for 3 minutes or until all beads are collected against the magnets.

e. Keep the plate on the magnet and carefully remove the seal, then transfer the eluates to a fresh standard (not deep-well) plate.

IMPORTANT! To prevent evaporation, seal the plate containing the eluate immediately after the transfers are complete.

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**.

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

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

Revision history: Pub. No. MAN0018069

Revision	Date	Description
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.