# MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit

## High throughput isolation of Nucleic Acid (RNA and DNA) from soil, biofluids, and other samples

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

Pub. No. MAN0018070 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 soil, swabs, and liquid samples (e.g. biofluids). 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 soil and liquid 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™ 96 Deep-Well 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 deep-well 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.
- *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 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 carefully 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 the Binding Bead Mix according to the following table:

| Component | Volume per well[1] 96 deep-well plates | Volume per well[1] 24 deep-well plates |
|---|---|---|
| Binding Solution | 500 µL | 2,500 µL |
| Total Nucleic Acid Binding Beads | 20 µL | 100 µL |
| **Total volume** | **520 µL** | **2,600 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 has been downloaded from the product page and loaded onto the instrument:
   - Soil Samples: **MagMAX_Microbiome_Soil_Flex**
   - Biofluid and other sample types: **MagMAX_Microbiome_Liquid_Buccal_Flex**

### 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 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.

4. Vortex the bead tube upside down for 10 seconds to mix the sample with the buffer.
5. Set the speed to 2,500 × *g*, 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.

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 | 50 µL |
| Tip Comb | 7 | Place a 96 Deep-well Tip Comb in a Standard deep-well Plate | | |

**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 Digest with Proteinase K

1. Remove tubes from vortexer, then centrifuge at 14,000 × *g* for 2 minutes.
2. 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.
3. Add 40 µL of Proteinase K to each sample.
4. Select the correct program on the instrument:
   - Soil Samples: **MagMAX_Microbiome_Soil_Flex**
   - Biofluid and other sample types: **MagMAX_Microbiome_Liquid_Buccal_Flex**
5. Start the run, then load the prepared plates into position when prompted by the instrument.

## 5 Bind, wash, then elute total nucleic acid

1. When prompted (approximately 20 minutes after the start of the protocol), remove the Sample Plate from the instrument.
2. 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.
3. Place the Sample Plate back onto the instrument, then start the run.
4. After the protocol is complete (~35 minutes after adding Binding Bead Mix), 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 has been downloaded from the product page and loaded onto the instrument:
   - Soil Samples: **MagMAX_Microbiome_Soil**
   - Biofluid and other sample types: **MagMAX_Microbiome_Liquid_Buccal**

### 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 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.

4. Vortex the bead tube upside down for 10 seconds to mix the sample with the buffer.
5. Set the speed to 2,500 × *g*, 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.

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 | 50 µL |
| Tip Comb | 1 | Place a 96 Deep-well Tip Comb in a Standard deep-well Plate | | |

**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 Digest with Proteinase K

1. Remove tubes from vortexer, then centrifuge at 14,000 × *g* for 2 minutes.
2. 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.
3. Add 40 µL of Proteinase K to each sample.
4. Select the correct program on the instrument:
   - Soil Samples: **MagMAX_Microbiome_Soil**
   - Biofluid and other sample types: **MagMAX_Microbiome_Liquid_Buccal**
5. Start the run, then load the prepared plates into position when prompted by the instrument.

## 5 Bind, wash, then elute total nucleic acid

1. When prompted (approximately 20 minutes after the start of the protocol), remove the Sample Plate from the instrument.
2. 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.
3. Place the Sample Plate back onto the instrument, then start the run.
4. After the protocol is complete (~35 minutes after adding Binding Bead Mix), 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 has been downloaded from the product page and loaded onto the instrument:
   - Soil Samples: **MagMAX_Microbiome_Soil_Flex**
   - Biofluid and other sample types: **MagMAX_Microbiome_Liquid_Buccal_Flex**

### 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 | 50 µL |
| Tip Comb | 7 | Place a 96 Deep-well Tip Comb in a Standard deep-well Plate | | |

**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 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 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.

## 3 *(continued)*

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 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 × *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 Digest with Proteinase K

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

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

2. 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.

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

4. Select the correct program on the instrument:
   - Soil Samples: **MagMAX_Microbiome_Soil_Flex**
   - Biofluid and other sample types: **MagMAX_Microbiome_Liquid_Buccal_Flex**

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

## 5 Bind, wash, then elute total nucleic acid

1. When prompted (approximately 20 minutes after the start of the protocol), remove the Sample Plate from the instrument.

2. 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.

3. Place the Sample Plate back onto the instrument, then start the run.

4. After the protocol is complete (~35 minutes after adding Binding Bead Mix), 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 has been downloaded from the product page and loaded onto the instrument:
   - Soil Samples: **MagMAX_Microbiome_Soil**
   - Biofluid and other sample types: **MagMAX_Microbiome_Liquid_Buccal**

### 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 | 50 µL |
| Tip Comb | 1 | Place a 96 Deep-well Tip Comb in a Standard deep-well Plate | | |

**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 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 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.

**3** *(continued)*

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 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 × *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** **Digest with Proteinase K**

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

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

2. 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.

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

4. Select the correct program on the instrument:
   - Soil Samples: **MagMAX_Microbiome_Soil**
   - Biofluid and other sample types: **MagMAX_Microbiome_Liquid_Buccal**

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

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

1. When prompted (approximately 20 minutes after the start of the protocol), remove the Sample Plate from the instrument.

2. 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.

3. Place the Sample Plate back onto the instrument, then start the run.

4. After the protocol is complete (~35 minutes after adding Binding Bead Mix), 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 has been downloaded from the product page and loaded onto the instrument:
   - Soil Samples: **MagMAX_Microbiome_Soil_Duo**
   - Biofluid and other sample types: **MagMAX_Microbiome_Liquid_Buccal_Duo**

### 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 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.

4. Vortex the bead tube upside down for 10 seconds to mix the sample with the buffer.
5. Set the speed to 2,500 × *g*, 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.

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 and Elution Strip

Set up the Sample Plate and Elution Strip according to the following tables, respectively.

**Table 2 Sample Plate**

| Row ID | Plate Row | Reagent | Volume per well |
|---|---|---|---|
| Sample row | A | Sample | Varies |
| 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 |
| — | H | Empty | |

**Table 3 Elution strip**

| Row ID | Plate Row | Reagent | Volume per well |
|---|---|---|---|
| Elution Solution | A | Elution Solution | 50 µL |

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

## 4 Digest with Proteinase K

1. Remove tubes from vortexer, then centrifuge at 14,000 × *g* for 2 minutes.
2. Transfer 400–500 µL of the sample to the appropriate wells of Row A in the Sample Plate.

   **Note:** For soil samples, transfer as much supernatant as possible without any particle carryover.
3. Add 40 µL of Proteinase K to each sample.
4. Select the correct program on the instrument:
   - Soil Samples: **MagMAX_Microbiome_Soil_Duo**
   - Biofluid and other sample types: **MagMAX_Microbiome_Liquid_Buccal_Duo**
5. Start the run, then load the prepared plate and elution strip into position when prompted by the instrument.

## 5 Bind, wash, then elute total nucleic acid

1. When prompted (approximately 20 minutes after the start of the protocol), remove the Sample Plate from the instrument.
2. Invert Binding Bead Mix to mix, then add 520 µL to each sample in Row A 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.
3. Place the Sample Plate back onto the instrument, then start the run.
4. After the protocol is complete (~35 minutes after adding Binding Bead Mix), immediately remove the elution strip 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 has been downloaded from the product page and loaded onto the instrument:
   - Soil Samples: **MagMAX_Microbiome_Soil_Duo**
   - Biofluid and other sample types: **MagMAX_Microbiome_Liquid_Buccal_Duo**

### 2 Set up the Sample Plate and Elution Strip

Set up the Sample Plate and Elution Strip according to the following tables, respectively.

**Table 4 Sample Plate**

| Row ID | Plate Row | Reagent | Volume per well |
|---|---|---|---|
| Sample row | A | Sample | Varies |
| 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 |
| — | H | Empty | |

**Table 5 Elution strip**

| Row ID | Plate Row | Reagent | Volume per well |
|---|---|---|---|
| Elution Solution | A | Elution Solution | 50 µL |

**Note:** To prevent evaporation and contamination, cover the prepared processing plate and elution strip 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 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 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 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 × *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 Digest with Proteinase K

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

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

2. Transfer 400–500 µL of the sample to the appropriate wells of Row A in the Sample Plate.

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

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

4. Select the correct program on the instrument:
   - Soil Samples: **MagMAX_Microbiome_Soil_Duo**
   - Biofluid and other sample types: **MagMAX_Microbiome_Liquid_Buccal_Duo**

5. Start the run, then load the prepared plate and elution strip into position when prompted by the instrument.

## 5 Bind, wash, then elute total nucleic acid

1. When prompted (approximately 20 minutes after the start of the protocol), remove the Sample Plate from the instrument.

2. Invert Binding Bead Mix to mix, then add 520 µL to each sample in Row A 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.

5 *(continued)*

3. Place the Sample Plate back onto the instrument, then start the run.

4. After the protocol is complete (~35 minutes after adding Binding Bead Mix), immediately remove the elution strip 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 its affiliates 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 questions, contact Life Technologies at **www.thermofisher.com/support**.

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| 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. |