MagMAX™ DNA Multi-Sample Ultra 2.0 Kit

With MagMAX™ DNA Cell and Tissue Extraction Buffer for high-throughput isolation of DNA from cultured cells

Catalog Number A45721

Pub. No. MAN0018808 Rev. B

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™ DNA Multi-Sample Ultra 2.0 Kit is developed for scalable, rapid purification of high-quality DNA from a variety of sample matrices. DNA purified with this kit can be used in a broad range of molecular biology downstream applications, such as sequencing, genotyping, and qPCR. This protocol describes automated isolation of DNA from cultured cells using the KingFisher™ Flex and the KingFisher™ Duo Prime instruments.

Contents and storage

Reagents provided in the kit are sufficient for 100 reactions using up to 1 × 10^6 cells per reaction.

Table 1 MagMAX™ DNA Multi-Sample Ultra 2.0 Kit

Component	Amount	Storage
Enhancer Solution	4.5 mL	15–30°C
Proteinase K	4.5 mL	
Binding Solution	45 mL	
DNA Binding Beads	4.5 mL	
Wash I Solution	110 mL	
Elution Solution[1]	12 mL	

[1] The amount of Elution Solution provided in the kit is sufficient for preparing 50 samples. If needed, additional Elution Solution can be purchased separately (Cat. No. A36582).

Table 2 MagMAX™ DNA Cell and Tissue Extraction Buffer

Component	Amount[1]	Storage
MagMAX™ DNA Cell and Tissue Extraction Buffer (Extraction Buffer)	60 mL	15–30°C

[1] Also available separately (Cat. No. A45469).

For 1,000 reactions use Cat. No. A36579 (DNA Binding Beads), A36580 (Wash I Solution), A36581 (Lysis/Binding Solution), 2 × A36582 (Elution Solution), and A45470 (MagMAX™ DNA Cell and Tissue Extraction Buffer).

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
Instrument	
Magnetic particle processor (one of the following, depending on the quantity/volume of sample to be processed):	
• KingFisher™ Duo Prime Purification System	5400110
• *For up to 1 × 10^6 cells*: KingFisher™ Flex Purification System with 96 Deep-Well Head	5400630
• *For up to 5 × 10^6 cells*: KingFisher™ Flex Purification System with 24 Deep-Well Head	5400640

Item	Source
Consumables	
Deep-well plates: • *For up to 1 × 10^6 cells*: KingFisher™ Deepwell 96 Plate • *For up to 5 × 10^6 cells*: KingFisher™ Flex 24 Deep-well plate	95040450 95040470
96-well standard plates (for use with KingFisher™ Flex only; tip comb placement and eluate storage): KingFisher™ 96 KF microplates (200 µL)	97002540
Tip comb, compatible with the magnetic particle processor used: • KingFisher™ Duo Prime 12-tip comb (for use with KingFisher™ Deepwell 96 Plate) • KingFisher™ Duo Prime 6-tip comb (for use with KingFisher™ Flex 24 Deep-well plate) • KingFisher™ 96 tip comb for DW magnets (KingFisher™ Flex protocol only) • KingFisher™ Flex 24 Deep Well Tip Comb and plate (KingFisher™ Flex protocol only)	97003500 97003510 97002534 97002610
Elution strip (KingFisher™ Duo Prime protocol only; elution step): • Elution strip • KingFisher™ Duo Cap for elution strip	97003520 97003540
Equipment	
Adjustable micropipette	MLS
Multichannel micropipette	MLS
Reagents	
PureLink™ RNase A (20 mg/mL) (RNase A)	12091021
Ethanol, 96–100% (molecular biology grade)	MLS
Nuclease-Free Water	AM9932
Materials	
MicroAmp™ Clear Adhesive Film	4306311

General guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Precipitates and high viscosity can occur if Enhancer Solution, Wash I Solution, and Binding Solution are stored when room temperature is too cold. If this occurs, warm them at 37°C, then gently mix to dissolve precipitates. Avoid creating bubbles.
- Yellowing of the Binding Solution and Wash I Solution is normal and will not affect buffer performance
- Per-plate volumes for reagent mixes are sufficient for one plate plus overage. To calculate volumes for other sample numbers, refer to the per-well volume and add 10% overage.
- *(Optional)*: 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.

Guidelines for DNA Binding Bead Mix

- Vortex the DNA Binding Beads thoroughly, combine them with the Binding Solution in a nuclease-free tube, then invert the tube until homogeneous. This mixture can be stored for up to 1 day before aliquoting into the plates.
- Ensure that the beads stay fully mixed within the solution during pipetting.
- Avoid creating bubbles during mixing and aliquoting.

Before first use of the kit

Note: RNase A is recommended for this protocol and must be purchased separately from the kit. See "Required materials not supplied" on page 1.

Prepare Wash II Solution: Make 80% ethanol from 100% absolute ethanol and nuclease-free water.

IMPORTANT! The Wash I Solution and Lysis/Binding Solution may develop inert white or brown particulates that float in the solution. Visual particulate is not a cause for concern and does not negatively affect performance.

Before each use of the kit

Vortex DNA Binding Beads to fully resuspend the beads before each use.

Perform DNA purification using KingFisher™ Flex (96 deep-well format)

This method is recommended for samples that contain up to 1 × 10^6 cultured cells.

1 Set up the instrument

1. Ensure that the instrument is set up for processing with the proper magnetic head (96 deep-well) for your application.
2. Ensure that the proper heat block (96 deep-well, not standard) is installed for your application.
3. Ensure that the proper program (**MMX_Ultra2_Cell_Tissue_96_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 of the instrument according to the following table.

Plate ID	Plate position	Plate type	Reagent	Volume per well
Up to 1 × 10^6 cell input				
Wash I Solution Plate	2	Deep Well	Wash I Solution	1,000 µL
Wash II Solution Plate 1	3	Deep Well	Wash II Solution	1,000 µL
Wash II Solution Plate 2	4	Deep Well	Wash II Solution	500 µL
Elution Plate	5	Deep Well	Elution Solution	200 µL
Tip Comb	6	Place a 96 Deep-well Tip Comb in a Standard Plate		

Note: Load the plates on the instrument immediately after the Sample Plate is prepared.

3 Prepare DNA Binding Bead Mix

Prepare DNA Binding Bead Mix according to the following table.

Component	Volume per well
Binding Solution	400 µL
DNA Binding Beads	40 µL
Total DNA Binding Bead Mix	**440 µL**

4 Prepare the Sample Plate

If needed, thaw the cell pellets at room temperature, then carefully remove as much media as possible from each pellet.

1. To each cell pellet, add 500 µL of Extraction Buffer for every 1 × 10^6 cells.
2. Vortex to dislodge the cell pellet from the tube.

   The cell pellet forms a clear, viscous, free-floating mass.
3. Using a P1000 pipette, pipet up and down 10 times or until the cell pellet is completely dissolved.

   IMPORTANT! The sample can become viscous. It is critical to pipet up and down thoroughly to ensure complete mixing.
4. Transfer each sample to the appropriate well of a deep-well plate (Sample Plate).

   Pipet slowly to ensure that the entire sample is transferred, including the viscous portion.
5. (Recommended) Add 10 µL of RNase A to each sample, seal the plate with adhesive film, then mix using a plate shaker set at moderate speed for at least 5 minutes at room temperature.

   Alternatively, if a plate shaker is not available, pipet up and down 10 times to mix, then incubate for at least 5 minutes at room temperature.
6. Invert the tube of DNA Binding Bead Mix several times to resuspend the beads, then add 440 µL of DNA Binding Bead Mix to each sample.

   Note:
   - Remix DNA Binding Bead Mix frequently during pipetting to ensure that the beads are evenly distributed among the samples.
   - The mixture is viscous. Pipet slowly to ensure that the correct amount is added to each sample.
7. Immediately proceed to process samples on the instrument (next section).

5 Process samples on the instrument

1. Select the **MMX_Ultra2_Cell_Tissue_96_Flex** program on the instrument.
2. Start the run, then load the prepared plates in their positions when prompted by the instrument.
3. At the end of the run, immediately remove the plate from the instrument, then transfer the eluate to a tube or plate for storage.

Store purified DNA on ice for immediate use, at –20°C for up to 1 month, or at –80°C for long-term storage.

Perform DNA purification using KingFisher™ Flex (24 deep-well format)

This method is recommended for samples that contain up to 5 × 10^6 cultured cells.

1 Set up the instrument

1. Ensure that the instrument is set up for processing with the proper magnetic head (24 deep-well) for your application.
2. Ensure that the proper heat block (24-well) is installed for your application.
3. Ensure that the proper program (**MMX_Ultra2_Cell_Tissue_24L_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 of the instrument according to the following table.

Plate ID	Plate position	Plate type	Reagent	Volume per well
Up to 5 × 10^6 cell input				
Wash I Solution Plate	2	Deep Well	Wash I Solution	5 mL
Wash II Solution Plate 1	3	Deep Well	Wash II Solution	4 mL
Wash II Solution Plate 2	4	Deep Well	Wash II Solution	2 mL
Elution Plate	5	Deep Well	Elution Solution	1 mL
Tip Comb	6	Place a 24 Deep-well Tip Comb in a Standard Plate		

Note: Load the plates on the instrument immediately after the Sample Plate is prepared.

3 Prepare DNA Binding Bead Mix

Prepare DNA Binding Bead Mix according to the following table.

Component	Volume per well
Binding Solution	2 mL
DNA Binding Beads	200 µL
Total DNA Binding Bead Mix	**2.2 mL**

4 Prepare the Sample Plate

If needed, thaw the cell pellets at room temperature, then carefully remove as much media as possible from each pellet.

1. To each cell pellet, add 2.5 mL of Extraction Buffer for every 5 × 10^6 cells.
2. Vortex to dislodge the cell pellet from the tube.

   The cell pellet forms a clear, viscous, free-floating mass.
3. Using a P1000 pipette, pipet up and down 10 times.
4. Incubate the sample for 5–10 minutes at room temperature.

4 *(continued)*

5. Vortex, then use a P1000 pipette to pipet up and down 10 times or until the cell pellet is completely dissolved.

   IMPORTANT! The sample can become viscous. It is critical to pipet up and down thoroughly to ensure complete mixing.

6. Transfer each sample to the appropriate well of a deep-well plate (Sample Plate).

   Pipet slowly to ensure that the entire sample is transferred, including the viscous portion.

7. (Recommended) Add 50 µL of RNase A to each sample, seal the plate with adhesive film, then mix using a plate shaker set at moderate speed for at least 10 minutes at room temperature.

   Alternatively, if a plate shaker is not available, pipet up and down 10 times to mix, then incubate for at least 10 minutes at room temperature.

8. Invert the tube of DNA Binding Bead Mix several times to resuspend the beads, then add 2.2 mL of DNA Binding Bead Mix to each sample.

   Note:
   - Remix DNA Binding Bead Mix frequently during pipetting to ensure that the beads are evenly distributed among the samples.
   - The mixture is viscous. Pipet slowly to ensure that the correct amount is added to each sample.

9. Immediately proceed to process samples on the instrument (next section).

5 **Process samples on the instrument**

1. Select the **MMX_Ultra2_Cell_Tissue_24L_Flex** program on the instrument.
2. Start the run, then load the prepared plates in their positions when prompted by the instrument.
3. At the end of the run, immediately remove the plate from the instrument, then transfer the eluate to a tube or plate for storage.

Store purified DNA on ice for immediate use, at –20°C for up to 1 month, or at –80°C for long-term storage.

Perform DNA purification using KingFisher™ Duo Prime (96 deep-well format)

This method is recommended for samples that contain up to 1×10^6 cultured cells.

1 **Set up the instrument**

1. Ensure that the instrument is set up for processing with the proper magnetic head (12 pin) and heat block for your application.
2. Ensure that the proper program (**MMX_Ultra2_Cell_Tissue_96_Duo**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plates

Prepare the 96 deep-well plate according to the following table.

Note: The Sample Row will be prepared in a later step.

Row ID	Plate row	Reagent	Volume per well
Plate layout			
Elution Solution	A	Elution Solution	200 µL
Tip Comb	B	Tip Comb	N/A
—	C	Empty	
Wash II Solution	D	Wash II Solution	500 µL
Wash II Solution	E	Wash II Solution	1,000 µL
Wash I Solution	F	Wash I Solution	1,000 µL
—	G	Empty	
Sample	H	Sample	Varies

Note: Load the plate on the instrument immediately after the Sample Row is prepared.

3 Prepare DNA Binding Bead Mix

Prepare DNA Binding Bead Mix according to the following table.

Component	Volume per well
Binding Solution	400 µL
DNA Binding Beads	40 µL
Total DNA Binding Bead Mix	**440 µL**

4 Prepare the Sample Row

If needed, thaw the cell pellets at room temperature, then carefully remove as much media as possible from each pellet.

1. To each cell pellet, add 500 µL of Extraction Buffer for every 1×10^6 cells.
2. Vortex to dislodge the cell pellet from the tube.

   The cell pellet forms a clear, viscous, free-floating mass.
3. Using a P1000 pipette, pipet up and down 10 times or until the cell pellet is completely dissolved.

   IMPORTANT! The sample can become viscous. It is critical to pipet up and down thoroughly to ensure complete mixing.
4. Transfer each sample to Row H (Sample Row) of the prepared deep-well plate.

   Pipet slowly to ensure that the entire sample is transferred, including the viscous portion.
5. (Recommended) Add 10 µL of RNase A to each sample, pipet up and down 10 times to mix, then incubate for at least 5 minutes at room temperature.
6. Invert the tube of DNA Binding Bead Mix several times to resuspend the beads, then add 440 µL of DNA Binding Bead Mix to each sample in Row H.

   Note:
   - Remix DNA Binding Bead Mix frequently during pipetting to ensure that the beads are evenly distributed among the samples.
   - The mixture is viscous. Pipet slowly to ensure that the correct amount is added to each sample.
7. Immediately proceed to process samples on the instrument (next section).

5 Process samples on the instrument

1. Select the **MMX_Ultra2_Cell_Tissue_96_Duo** program on the instrument.
2. Start the run, then load the prepared plates in their positions when prompted by the instrument.
3. At the end of the run, immediately remove the plate from the instrument, then transfer the eluate to a tube or plate for storage.

Store purified DNA on ice for immediate use, at –20°C for up to 1 month, or at –80°C for long-term storage.

Perform DNA purification using KingFisher™ Duo Prime (24 deep-well format)

This method is recommended for samples that contain up to 5 × 10^6 cultured cells.

1 Set up the instrument

1. Ensure that the instrument is set up for processing with the proper magnetic head (6 pin) and heat block for your application.
2. Ensure that the proper program (**MMX_Ultra2_Cell_Tissue_24L_Duo**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plates

Prepare the 24 deep-well plates according to the following table.

Note: The Sample Row will be prepared in a later step.

Row ID	Plate row	Reagent	Volume per well
Plate 1 layout			
Sample	A	Sample	Varies
Wash I Solution	B	Wash I Solution	5 mL
Wash II Solution	C	Wash II Solution	4 mL
Wash II Solution	D	Wash II Solution	2 mL
Plate 2 layout			
Elution Solution	A	Elution Solution	1 mL
Tip Comb	B	Tip Comb	N/A
—	C	Empty	
—	D	Empty	

Note: Load the plates on the instrument immediately after the Sample Row is prepared.

3 Prepare DNA Binding Bead Mix

Prepare DNA Binding Bead Mix according to the following table.

Component	Volume per well
Binding Solution	2 mL
DNA Binding Beads	200 µL
Total DNA Binding Bead Mix	**2.2 mL**

4 Prepare the Sample Row

If needed, thaw the cell pellets at room temperature, then carefully remove as much media as possible from each pellet.

1. To each cell pellet, add 2.5 mL of Extraction Buffer for every 5 × 10^6 cells.
2. Vortex to dislodge the cell pellet from the tube.

   The cell pellet forms a clear, viscous, free-floating mass.

4 *(continued)*

3. Using a P1000 pipette, pipet up and down 10 times.
4. Incubate the sample for 5–10 minutes at room temperature.
5. Vortex, then use a P1000 pipette to pipet up and down 10 times or until the cell pellet is completely dissolved.

   IMPORTANT! The sample can become viscous. It is critical to pipet up and down thoroughly to ensure complete mixing.

6. Transfer each sample to Row A (Sample Row) of the prepared Plate 1.

   Pipet slowly to ensure that the entire sample is transferred, including the viscous portion.

7. (Recommended) Add 50 µL of RNase A to each sample, pipet up and down 10 times to mix, then incubate for at least 10 minutes at room temperature.
8. Invert the tube of DNA Binding Bead Mix several times to resuspend the beads, then add 2.2 mL of DNA Binding Bead Mix to each sample.

   Note:
   - Remix DNA Binding Bead Mix frequently during pipetting to ensure that the beads are evenly distributed among the samples.
   - The mixture is viscous. Pipet slowly to ensure that the correct amount is added to each sample.

9. Immediately proceed to process samples on the instrument (next section).

5 **Process samples on the instrument**

1. Select the **MMX_Ultra2_Cell_Tissue_24L_Duo** program on the instrument.
2. Start the run, then load the prepared plates in their positions when prompted by the instrument.
3. At the end of the run, immediately remove the plate from the instrument, then transfer the eluate to a tube or plate for storage.

Store purified DNA on ice for immediate use, at –20°C for up to 1 month, or at –80°C for long-term storage.

Quantitate the gDNA samples

To most accurately quantitate gDNA samples that are isolated from cultured cells, we recommend using a NanoDrop™ spectrophotometer. Quantitation can also be performed using qPCR and the Applied Biosystems™ TaqMan™ RNase P Detection Reagents Kit (Cat. No. 4316831).

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.

Revision history: Pub. No. MAN0018808 B

Revision	Date	Description
B	26 July 2024	• Corrected the product catalog number. • Important statement added to the user guide under the section "Before first use of the kit".
A.0	7 October 2019	New document for new sample type.

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