MagMAX™ DNA Multi-Sample Ultra 2.0 Kit

High throughput isolation of DNA from buccal swabs

Catalog Number A36570

Pub. No. MAN0017205 Rev. E

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 guides through automated isolation of DNA from buccal swabs using the KingFisher™ Flex, KingFisher™ Apex, and the KingFisher™ Duo Prime.

Contents and storage

Reagents provided in the kit are sufficient for 100 reactions.

Table 1 MagMAX™ DNA Multi-Sample Ultra 2.0 Kit (Cat. No. A36570)

Component	Quantity	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	12 mL	

For 1,000 reaction volume use Cat. No. A36578 (Proteinase K), A36579 (DNA Binding Beads), A36580 (Wash I Solution), A36581 (Lysis/Binding Solution), A36582 (Elution Solution), and A36583 (Enhancer Solution).

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.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Instrument	
Magnetic particle processor, one of the following:	
KingFisher™ Duo Prime Magnetic Particle Processor	5400110
KingFisher™ Flex Magnetic Particle Processor 96DW with 96 deep-well head	5400630
KingFisher™ Apex with 96 deep–well head	5400930
Consumables	
Deep-well plates, one of the following:	
KingFisher™ 96 Deep-Well Plates, v-bottom, polypropylene	95040450
KingFisher™ 96 Deep-Well Plates, barcoded	95040450B
96-well standard plates (for use with KingFisher™ Flex and KingFisher™ Apex only; elution step):	
KingFisher™ 96 KF microplate	97002540
Tip comb, compatible with the magnetic particle processor used:	
KingFisher™ 12-tip comb, for 96 deep-well plate (KingFisher™ Duo Prime protocols only)	97003500
KingFisher™ 96 tip comb for DW magnets (KingFisher™ Flex and KingFisher™ Apex protocols only)	97002534
Equipment	
Incubator with metal racks	MLS
Plate shaker, capable of shaking plates at a minimum of 900 rpm	88880023
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS

Item	Source
Reagents	
Ethanol, 96–100% (molecular biology grade)	MLS
Nuclease-free water	AM9932
Phosphate buffered saline (PBS (1X), pH 7.4)	10010023
Materials	
MicroAmp™ Clear Adhesive Film	4306311
4N6FLOQSwabs™, regular tip	4473979

General guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Precipitates and high viscosity can occur if Enhancer Solution and Binding Solution are stored when room temperature is too cold. If this occurs, warm them at 37°C and gently mix to dissolve precipitates and reduce viscosity. Avoid creating bubbles.
- Yellowing of the Binding and Wash I Solution is normal and will not affect buffer performance
- Cover the plate during the incubation and shaking steps to prevent spill-over and cross-contamination. The same MicroAmp™ Clear Adhesive Film can be used throughout the procedure, unless it becomes contaminated.
- If using a plate shaker other than the recommended shaker, verify that:
  a. The plate fits securely on the plate shaker.
  b. The recommended speeds are compatible with the plate shaker (Ideal shaker speeds allow for thorough mixing without splashing).
- 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.

Sample collection and storage

IMPORTANT! Use the recommended swab with a foam tip. Use of cotton or generic polyester swabs may result in lower DNA yields or DNA that contains PCR inhibitors.

1. Have test subjects thoroughly rinse their mouths with water and swallow prior to swabbing.
2. Remove swab from packaging and thoroughly swab both cheeks of the test subject for 30 seconds each to maximize collection of buccal cells.
3. If necessary, store buccal swabs in the original pouch.

   IMPORTANT! Do not store buccal swabs in plastic tubes. Bacterial growth in sealed plastic tubes can cause DNA degradation.

Buccal swabs can be stored for up to 3 weeks at –20°C to 20°C before isolation.

Before first use of the kit

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.

Prepare samples and digest with Proteinase K

1 Prepare samples and digest with Proteinase K

Equilibrate buccal swabs to room temperature, before performing isolation, to maximize DNA recovery.

1. Place one swab, swab-head down, into a deep-well plate.

   Note: When a higher concentration of DNA is required, process two swabs in one well and proceed with the isolation as indicated.

1 *(continued)*

2. Break enough of the stick off the swab so that the swab sits in the well without protruding.

   The recommended swabs have an easy break point, below the swab, that appears as a slight indentation in the stick portion of the swab.

3. Prepare sufficient Proteinase K Mix according to the following table, then gently invert or pipet up and down several times to thoroughly mix components.

Component [1]	Volume per well	Volume per plate (96 samples)
Enhancer Solution	40 µL	4.22 mL
PBS	400 µL	42.24 mL
Proteinase K	40 µL	4.22 mL
Total volume	**480 µL**	**50.68 mL**

[1] Pipet the components in the order they are listed in the table.

IMPORTANT! Only make enough Mix for immediate use. Mix is not stable for prolonged periods and will result in a reduction of DNA yield.

4. Add 480 µL of the Proteinase K Mix to each well containing a swab.

   Be careful to avoid touching the pipette tip to the swab when pipetting the Proteinase K Mix into the sample wells.

5. Seal the plate with the clear adhesive film, then shake the sealed plate at 900 rpm for 5 minutes.

6. Take the plate off the plate shaker, then immediately incubate at 65°C for ≥ 20 minutes.

IMPORTANT! Arrange plate in the incubator to allow adequate flow around the plate wells to ensure that samples quickly reach and maintain the incubation temperature.

During the incubation, proceed with instrument and plate set up:

- For automated purification using the KingFisher™ Flex Magnetic Particle Processor, proceed to "Perform DNA purification using KingFisher™ Flex" on page 3.
- For automated purification using the KingFisher™ Apex instrument, proceed to "Perform DNA purification using KingFisher™ Apex" on page 5.
- For automated purification using the KingFisher™ Duo Prime Magnetic Particle Processor, proceed to "Perform DNA purification using KingFisher™ Duo Prime" on page 7.

Perform DNA purification using KingFisher™ Flex

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 well standard, not deep-well) is installed for your application.

3. Ensure that the proper program (**MagMAX_Ultra2_Direct_FLEX**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plates

During the incubation at 65°C, 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 I Solution Plate	2	Deep Well	Wash I Solution	1000 µL
Wash II Solution Plate 1	3	Deep Well	Wash II Solution	1000 µL
Wash II Solution Plate 2	4	Deep Well	Wash II Solution	500 µL
Elution Plate	5	Standard	Elution Solution	50 µL
Tip Comb	6	Place a 96 Deep-well Tip Comb in a Standard Plate		

Note: The plates will be loaded onto the instrument immediately after the Sample Plate has been prepared.

3 Bind the gDNA

1. Prepare the DNA Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate (96 samples)
Binding Solution	400 µL	42.24 mL
DNA Binding Beads	40 µL	4.22 mL
Total volume	**440 µL**	**46.46 mL**

2. At the end of Proteinase K digestion, shake the sealed plate at 900 rpm for 2 minutes.
3. Transfer the lysates to the corresponding wells of a new deep-well plate (this will be called the Sample Plate), then discard the buccal swabs.

To remove buccal swab from the lysate	Procedure
Transfer the lysate to a new plate.	1. Set a multi-channel pipettor to 480 µL and transfer one row at a time. 2. Ensure each well contains 420–480 µL after transfer.

4. Add 440 µL of DNA Binding Bead Mix to each sample.

   Note: Remix Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. Mixture is viscous, pipet slowly to ensure that the correct amount is added.

   IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

5. Immediately process the plates on the KingFisher™ Flex Magnetic Particle Processor 96DW.

4 Wash and elute the gDNA

1. Select the program **MagMAX_Ultra2_Direct_FLEX** on the instrument.
2. Start the run, and load the prepared plates into position when prompted by the instrument.
3. At the end of the run, immediately remove the plates from the instrument.
4. Transfer the eluate to the final tube/plate of choice for final storage.

   Note: If preferred, the elution plate may be used for final storage of the DNA.

The purified DNA is ready for immediate use. Alternatively, store the plate at –20°C for long-term storage.

Perform DNA purification using KingFisher™ Apex

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 both heat blocks (96 well standard and 96 deep–well) are installed on the KingFisher™ Apex instrument.
3. Ensure that the proper program (**MagMAX_Ultra2_Direct**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plates

During the incubation at 65°C, 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 I Solution Plate	3	Deep Well	Wash I Solution	1000 µL
Wash II Solution Plate 1	4	Deep Well	Wash II Solution	1000 µL
Wash II Solution Plate 2	5	Deep Well	Wash II Solution	500 µL
Elution Plate	6	Standard	Elution Solution	50 µL
Tip Comb	1	Place a 96 Deep-well Tip Comb in a Standard Plate		

Note: The plates will be loaded onto the instrument immediately after the Sample Plate has been prepared.

3 Bind the gDNA

1. Prepare the DNA Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate (96 samples)
Binding Solution	400 µL	42.24 mL
DNA Binding Beads	40 µL	4.22 mL
Total volume	**440 µL**	**46.46 mL**

2. At the end of Proteinase K digestion, shake the sealed plate at 900 rpm for 2 minutes.
3. Transfer the lysates to the corresponding wells of a new deep-well plate (this is the Sample Plate), then discard the buccal swabs.

To remove buccal swab from the lysate	Procedure
Transfer the lysate to a new plate.	1. Set a multi-channel pipettor to 480 µL and transfer one row at a time. 2. Ensure each well contains 420–480 µL after transfer.

4. Add 440 µL of DNA Binding Bead Mix to each sample.

   Note: Remix Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. Mixture is viscous, pipet slowly to ensure that the correct amount is added.

   IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

5. Immediately process the plates on the KingFisher™ Apex instrument.

4 Wash and elute the gDNA

1. Select the program **MagMAX_Ultra2_Direct** on the instrument.
2. Start the run, and load the prepared plates into position when prompted by the instrument.
3. At the end of the run, immediately remove the plates from the instrument.
4. Transfer the eluate to the final tube/plate of choice for final storage.

   Note: If preferred, the elution plate may be used for final storage of the DNA.

The purified DNA is ready for immediate use. Alternatively, store the plate at –20°C for long-term storage.

Perform DNA purification using KingFisher™ Duo Prime

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 (**MagMAX_Ultra2_Direct_DUO**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plate

Add processing reagents to the wells of the 96-well plate according to the following table.

Row ID	Plate Row	Reagent	Volume per well
Plate layout			
Elution Solution	A	Elution Solution	50–100 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	1000 µL
Wash I Solution	F	Wash I Solution	1000 µL
—	G	Empty	
Sample	H	Sample[1]	Varies

[1] See "Bind the gDNA" on page 7.

Note: The plate will be loaded onto the instrument immediately after the Sample Row has been prepared.

3 Bind the gDNA

1. Prepare the DNA Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate (12 samples)
Binding Buffer	400 µL	5.28 mL
DNA Binding Beads	40 µL	528 µL
Total volume	**440 µL**	**5.81 mL**

2. At the end of Proteinase K digestion, shake the sealed plate at 900 rpm for 2 minutes.
3. Transfer the lysates to the corresponding wells (Row H) of the previously prepared processing plate, then discard the buccal swabs.

To remove buccal swab from the lysate	Procedure
Transfer the lysate to a new plate.	1. Set a multi-channel pipettor to 480 µL and transfer one row at a time. 2. Ensure each well contains 420–480 µL after transfer.

4. Add 440 µL of DNA Binding Bead Mix to each sample (Row H).

   Note: Remix Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. Mixture is viscous, pipet slowly to ensure correct amount is added.

   IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

5. Immediately process the plate on the KingFisher™ Duo Prime Magnetic Particle Processor.

4 Wash and elute the gDNA

1. Select the program **MagMAX_Ultra2_Direct_DUO** on the instrument.
2. Start the run, and load the prepared plate into position when prompted by the instrument.
3. At the end of the run, immediately remove the plate from the instrument.
4. Transfer the eluate to the final tube/plate of choice for final storage.

The purified DNA is ready for immediate use. Alternatively, store the samples at –20°C for long-term storage.

Quantitation

To most accurately quantitate gDNA samples isolated from buccal swabs, it is recommended to quantitate using either the Qubit™ dsDNA BR (Broad Range) Assay Kit (Cat. No. Q32850) or Qubit™ dsDNA HS (High Sensitivity) Assay Kit (Cat. No. Q32851). Another acceptable method is quantitation utilizing qPCR and the Applied Biosystems™ TaqMan™ RNase P Detection Reagents Kit (Cat. No. 4316831).

Limited product warranty

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

Revision history: Pub. No. MAN0017205 E

Revision	Date	Description
E	31 July 2024	Important statement added to the user guide under the section "Before first use of the kit".
D.0	12 April 2021	Support added for KingFisher™ Apex Purification System.
C.0	15 March 2019	Updated manufacturing address to Vilnius. Added note to address yellowing buffers and viscosity concerns.
B.0	6 December 2017	Addition of quantitation section, and minor edits.
A.0	8 September 2017	New document for theMagMAX™ DNA Multi-Sample Ultra 2.0 Kit.