applied biosystems — USER GUIDE

MagMAX™ DNA Multi-Sample Ultra Kit

High-throughput isolation of PCR-ready DNA from buccal swabs

Catalog Numbers A25597 and A25598

Pub. No. MAN0010293 **Rev.** D.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 information

The MagMAX™ DNA Multi-Sample Ultra Kit is designed for rapid, high-throughput isolation of high-quality genomic DNA from a variety of sample matrices. The kit uses MagMAX™ magnetic bead technology, ensuring reproducible recovery of PCR-ready DNA suitable for a broad range of applications, such as SNP genotyping and copy number experiments.

These protocols describe isolation of DNA from buccal swabs, optimized for use with the KingFisher™ Flex Magnetic Particle Processor (96-well deep well setting) or the KingFisher™ Duo Prime Magnetic Particle Processor. The typical DNA yield obtained from 1 buccal swab is 2–12 µg at a concentration suitable for OpenArray™ analysis.

Kit contents and storage

Component	Cat. No. A25597[1] (500 rxns)	Cat. No. A25598[2] (2,500 rxns)	Storage
Proteinase K[3]	4 mL	5 × 4 mL	–25°C to –15°C
PK Buffer	96 mL	5 × 96 mL	15°C to 30°C
Multi-Sample DNA Lysis Buffer	100 mL	5 × 100 mL	15°C to 30°C
RNase A[4]	2 × 1.25 mL	10 × 1.25 mL	–25°C to –15°C
DNA Binding Beads[3]	8 mL	5 × 8 mL	2°C to 8°C
Nuclease-free Water	100 mL	5 × 100 mL	15°C to 30°C
Wash Solution 1 Concentrate	80 mL[5]	5 × 80 mL[5]	15°C to 30°C
Wash Solution 2 Concentrate	162 mL[5]	5 × 162 mL[5]	15°C to 30°C
DNA Elution Buffer 1	25 mL	5 × 25 mL	15°C to 30°C
DNA Elution Buffer 2	25 mL	5 × 25 mL	15°C to 30°C

[1] Also available as Cat. No. A25919, containing Cat. No. A25597 with **one additional tube** each of Proteinase K (4 mL) and DNA Binding Beads (8 mL).
[2] Also available as Cat. No. A25920, containing Cat. No. A25598 with **5 additional tubes** of Proteinase K (4 mL each) and **5 additional tubes** of DNA Binding Beads (8 mL each).
[3] Proteinase K is also available as Cat. no. A25561 and DNA Binding Beads are also available as Cat. No. A25562.
[4] Not used for DNA isolation from buccal swabs.
[5] Final volume; see "Before first use of the kit" on page 2.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Table 2 Required materials and equipment not included with the kit

Item	Source
Magnetic particle processor, one of the following:	
KingFisher™ Flex Magnetic Particle Processor	5400630
KingFisher™ Duo Prime Magnetic Particle Processor	5400110
Equipment	
Plate shaker, capable of shaking plates at a minimum of 900 rpm	88880023
Analog Vortex Mixer	Fisher Scientific 02-215-365
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
(*Optional*) Magnetic Stand-96	AM10027
Plates and combs	
96 deep-well plates, one of the following:	
KingFisher™ Deepwell 96 Plate, V-bottom, polypropylene	95040450
MagMAX™ Express-96 Deep Well Plates	4388476
96-well standard plates, one of the following:	
KingFisher™ 96 KF microplates	97002540
MagMAX™ Express-96 Standard Plates	4388475
Tip comb, compatible with the magnetic particle processor used:	
KingFisher™ 96 tip comb for DW magnets	97002534
MagMAX™ Express-96 Deep Well Tip Combs[1]	4388487
KingFisher™ Duo 12-Tip Comb, for Microtiter 96 Deepwell plate	97003500
Other consumables	
(For KingFisher™ Duo Prime Magnetic Particle Processor) KingFisher™ Duo Elution Strip	97003520
(For KingFisher™ Duo Prime Magnetic Particle Processor) KingFisher™ Duo Cap for elution strip	97003540
Other consumables	
MicroAmp™ Clear Adhesive Film	4306311
RNase-free Microfuge Tubes (2.0 mL)	AM12425
Conical tubes (15 mL)	AM12500
Conical tubes (50 mL)	AM12502
Aerosol-resistant pipette tips	MLS
Reagent reservoirs	MLS
(*Optional*) Paraffin film	MLS
Reagents	
Ethanol, 200 proof (absolute)	MLS
Isopropanol, 100% (molecular grade or higher)	MLS

[1] Compatible with the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head.

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Table 3 Additional materials and equipment required for processing buccal swabs

Item	Source
Laboratory incubator with slatted shelves, capable of maintaining 65°C	MLS
One of the following types of buccal swabs, or equivalent buccal swabs with foam tips:	
Puritan™ PurFlock™ Ultra Flocked Swabs	Fisher Scientific 22-025-192
Puritan™ HydraFlock™ Swabs, standard tip	Puritan 25–3306–H
Sterile Foam Tipped Swabs	Puritan 25-1506 1PF
4N6FLOQSwabs™, regular tip	4473979
(*Optional*) Proteinase K, 500 reactions (4 mL)	A25561
(*Optional*) DNA Binding Beads, 500 reactions (8 mL)	A25562

Sample collection and storage

- Use one of the following polyester swabs with foam tips. Use of cotton or generic polyester swabs may result in lower DNA yields or DNA that contains PCR inhibitors.
  - Puritan™ PurFlock™ Ultra Flocked Swabs (Fisher Scientific, Cat. no. 22-025-192)
  - Puritan™ HydraFlock™ Swabs, standard tip (Puritan, Cat. no. 25–3306–H)
  - Sterile Foam Tipped Swabs (Puritan, Cat. no. 25-1506 1PF)
  - 4N6FLOQSwabs™, regular tip (Cat. no. 4473979)
- Sample collection: Test subjects should thoroughly rinse their mouths with water and swallow prior to swabbing. Remove swab from packaging and thoroughly swab both cheeks of the test subject for 30 seconds each to maximize collection of buccal cells.
- Store buccal swabs in the original pouch. Do not use plastic tubes. Bacterial growth in sealed plastic tubes can cause DNA degradation.
- Buccal swab samples can be safely stored for up to 3 weeks at –20°C to room temperature before extraction with the MagMAX™ DNA Multi-Sample Ultra Kit.
- *(Optional)* Sample shipping: Ship dried buccal swabs after sample collection at 25°C or below.

Guidelines for buccal swab preparation

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Equilibrate buccal swabs to room temperature to maximize DNA recovery.
- Remove the buccal swabs from the lysate:
  - Option 1 (Preferred): Transfer the lysate to a new plate.

    This option eliminates contamination risks and saves time. To transfer lysates, set a multi-channel micropipettor to ~300 µL and transfer one row at the time. Each well should contain 200–250 µL after transfer.
  - Option 2: Remove the swabs from the plate using forceps.

    Rinse the forceps in 70% ethanol between samples, to prevent cross-contamination. Press the swabs against the side of the well when removing them, to prevent sample loss.
- 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 you use a plate shaker other than the recommended shaker, verify that:
  - The plate fits securely on your plate shaker.
  - The recommended speeds are compatible with your 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 5% overage.
- (*Optional*) To prevent evaporation and contamination, cover the prepared processing plates with paraffin film until they are loaded into the instrument.

(*Optional*) Guidelines for improving yields

- This procedure is optimized for processing of one swab per well. It is possible to process two swabs in one well when a higher concentration of DNA is required.
- If the DNA yield is lower than expected, extend the Proteinase K digestion to 45 minutes.
- To further improve recovery, digest with Proteinase K overnight at 50°C.

Before first use of the kit

Prepare the Wash Solutions from the concentrates:

- Add 25 mL of isopropanol to Wash Solution 1 Concentrate, mix, and store at room temperature.
- Add 132 mL of ethanol to Wash Solution 2 Concentrate, mix, and store at room temperature.

Before each use of the kit

- Preheat the incubator to 65°C.
- Vortex DNA Binding Beads thoroughly, then combine with Nuclease-free Water according to the following table.

Component	Volume per well
DNA Binding Beads	16 µL
Nuclease-free Water	24 µL
Total DNA Binding Bead Mix	40 µL

Store DNA Binding Bead Mix at room temperature for up to 24 hours.

Digest the samples with Proteinase K

Ensure that the incubator is preheated to 65°C.

1. Place the swab, swab-head down, in a deep-well plate (one per well).

   If you are using the KingFisher™ Duo Prime Magnetic Particle Processor, make sure to place all the swabs in the wells of the same row.

   If two swabs were collected, store the second swab as a backup sample.
2. Break enough of the stick off the swabs so that the swabs sit in the wells without protruding.
3. Prepare sufficient PK Mix according to the following table, then invert several times to thoroughly mix components.

   IMPORTANT! Prepare the PK Mix just before use. Do not place the PK Buffer or the PK Mix on ice, to avoid precipitation.

Component	Volume per well
Proteinase K	8 µL
PK Buffer	192 µL
Total PK Mix	**200 µL**

4. Add 200 µL of PK Mix to each sample well of a deep-well plate (PK Plate) containing a swab.

   IMPORTANT! Do not touch any part of the swab with the pipet tip when pipetting the PK Mix in the sample wells.
5. Seal the plate with a clear adhesive film, then shake the sealed plate at 900–950 rpm for 5 minutes.
6. Incubate for 20 minutes at 65°C.

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

Proceed with the DNA purification.

- For automated purification using KingFisher™ Flex Magnetic Particle Processor 96DW, proceed to "Perform DNA extraction and elution using KingFisher™ Flex Magnetic Particle Processor" on page 3.
- For automated purification using KingFisher™ Duo Prime Magnetic Particle Processor, proceed to "Perform DNA extraction and elution using KingFisher™ Duo Prime Magnetic Particle Processor" on page 4.

Perform DNA extraction and elution using KingFisher™ Flex Magnetic Particle Processor

1 Set up the processing plates

a. While the samples are incubating at 65°C, set up the Wash, Elution, and Tip Comb Plates outside the instrument as described in the following table.

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well
Wash Plate 1	2	Deep Well	Wash Solution 1	150 µL
Wash Plate 2	3	Deep Well	Wash Solution 2	150 µL
Wash Plate 3	4	Deep Well	Wash Solution 2	150 µL
Elution Plate[2]	5	Standard	DNA Elution Buffer 1	50 µL
Tip Comb	6	Deep Well	Place a tip comb in the plate.	

[1] Position on the instrument

[2] The instrument prompts the user to add DNA Elution Buffer 2 to the Elution Plate, after incubation with DNA Elution Buffer 1.

b. (*Optional*) To prevent evaporation and contamination, cover the prepared processing plates with paraffin film until they are loaded into the instrument.

2 Add Multi-Sample DNA Lysis Buffer, isopropanol, and DNA Binding Bead Mix

a. If condensation is present at the end of the 65°C incubation, briefly centrifuge the plate at 1,500 × *g* for 1–2 minutes.

b. Add 200 µL of Multi-Sample DNA Lysis Buffer to each sample.

c. Seal the plate with a clear adhesive film, then shake at 900–950 rpm for 5 minutes.

d. Transfer lysates to the wells of a new deep-well plate and discard the plate containing the buccal swabs.

e. Add 240 µL of isopropanol to each sample, seal the plate, then shake at 900–950 rpm for 5 minutes.

f. Vortex DNA Binding Bead Mix at moderate speed to ensure thorough mixing, add 40 µL to each sample, then proceed immediately to DNA isolation (next section).

If you see that the beads in the DNA Binding Bead Mix are settling, vortex the mix again briefly before continuing to pipette.

3 Process samples on the instrument

a. Select the **A25597_Blood_Buccal** on the instrument.

b. Start the run, remove the temporary paraffin plate seals (if present), then load the prepared processing plates in their positions when prompted by the instrument.

c. Load the PK plate (containing lysate, isopropanol, and DNA Binding Bead Mix) at position 1 when prompted by the instrument.

d. When prompted by the instrument (approximately 28–30 minutes after initial start):

1. Remove the Elution Plate from the instrument.

3 Process samples on the instrument *(continued)*

2. Add 50 µL of DNA Elution Buffer 2 to each sample well.

   IMPORTANT! Add DNA Elution Buffer 2 immediately after the prompt, to prevent excessive drying of any beads that are still captured on the Tip Comb.

3. Load the Elution Plate back onto the instrument, and press **Start**.

e. At the end of the run (approximately 30–35 minutes after initial start), remove the Elution Plate from the instrument and seal immediately with a new clear adhesive film.
   - (*Optional*) Eluates can be transferred to a new storage plate after collection.
   - If precipitated DNA is visible, pipet up and down 5–10 times before sealing the plate, to ensure complete resuspension.
   - If you see excessive bead residue in the wells, place the Elution Plate on the Magnetic Stand-96 to capture any residue prior to downstream use of the DNA.

   IMPORTANT! Do not allow the purified samples to sit uncovered at room temperature for more than 10 minutes, to prevent evaporation and contamination.

The purified samples are ready for immediate use. Alternatively, store the covered Elution Plate:
- At 2–6°C for up to 24 hours.
- At –20°C or –80°C for long-term storage.

Perform DNA extraction and elution using KingFisher™ Duo Prime Magnetic Particle Processor

1 Add Multi-Sample DNA Lysis Buffer, isopropanol, and DNA Binding Bead Mix

a. If condensation is present at the end of the 65°C incubation, briefly centrifuge the plate at 1,500 × *g* for 1–2 minutes.
b. Add 200 µL of Multi-Sample DNA Lysis Buffer to each sample.
c. Seal the plate with a clear adhesive film, then shake at 900–950 rpm for 5 minutes.
d. Transfer lysates to the corresponding wells in Row H of a new deep-well plate.
e. Add 240 µL of isopropanol to each sample, seal the plate, then shake at 900–950 rpm for 5 minutes.
f. Vortex DNA Binding Bead Mix at moderate speed to ensure thorough mixing, add 40 µL to each sample, then proceed immediately to next section.

   If you see that the beads in the DNA Binding Bead Mix are settling, vortex the mix again briefly before continuing to pipette.

2 Add processing reagents and set up the Elution Strip

a. Add processing reagents to the wells of the 96-well plate as indicated in the following table.

Table 4 Processing plate setup

Row ID	Plate row	Reagent	Volume per well
—	A	Empty	
Tip Comb	B	Place a KingFisher™ Duo 12-Tip Comb in Row B.	
—	C	Empty	
Wash Solution 2	D	Wash Solution 2	150 µL
Wash Solution 2	E	Wash Solution 2	150 µL
—	F	Empty	
Wash Solution 1	G	Wash Solution 1	150 µL
Sample	H	Sample lysate from previous section	

b. Prepare the Elution Strip as indicated in the following table.

Table 5 Elution Strip setup

Consumable	Reagent	Volume per well
KingFisher™ Duo Elution Strip	DNA Elution Buffer 1[1]	50 µL

[1] The instrument prompts to add DNA Elution Buffer 2 to the Elution Strip after incubation with DNA Elution Buffer 1

3 Process samples on the instrument

a. Ensure that the instrument is set up for processing with the 12–pin magnetic head and has the 12–well heating block installed.
b. Select the **A25597_Blood_Buccal** on the instrument.
c. Start the run, then load the prepared plate and the Elution Strip in their positions when prompted by the instrument.
d. When prompted by the instrument (approximately 28–30 minutes after initial start):
   1. Remove the Elution Strip from the instrument.
   2. Add 50 µL of DNA Elution Buffer 2 to each sample.

      IMPORTANT! Add DNA Elution Buffer 2 immediately after the prompt, to prevent excessive drying of any beads that are still captured on the Tip Comb.

   3. Load the Elution Strip back onto the instrument, and press **Start**.
e. At the end of the run (approximately 30–35 minutes after initial start), remove the Elution Strip from the instrument and cover immediately with a strip cap.
   - (*Optional*) Eluates can be transferred to a new storage plate after collection.

3	Process samples on the instrument *(continued)*	• If precipitated DNA is visible, pipet up and down 5–10 times before sealing the plate, to ensure complete resuspension. • If you see excessive bead residue in the wells, place the Elution Plate on the Magnetic Stand-96 to capture any residue prior to downstream use of the DNA. IMPORTANT! Do not allow the purified samples to sit uncovered at room temperature for more than 10 minutes, to prevent evaporation and contamination.

The purified samples are ready for immediate use. Alternatively, store the covered Elution Plate:

- At 2–6°C for up to 24 hours.
- At –20°C or –80°C for long-term storage.

Recommended quantitation methods

Standard curve analysis is the most accurate quantitation method, whereas UV absorbance measurements can be used to assess both the concentration and the quality of the isolated DNA.

- **Standard curve analysis.** Use the TaqMan® RNase P Copy Number Reference Assay (Cat. no. 4403326) for human genomic DNA and the TaqMan® DNA Template Reagents (Cat. no. 401970) to create a standard curve. Refer to *Creating Standard Curves with Genomic DNA or Plasmid DNA Templates for Use in Quantitative PCR* (Pub. no. 4371090).
- **UV absorbance measurements.** Use a NanoDrop™ or other comparable instrument. Pure genomic DNA should have an A_{260}/A_{280} ratio of approximately 1.6–2.0.

Note: Mix the samples by pipetting up and down before quantitation, if they have been stored frozen.

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Revision history: Pub. No. MAN0010293

Revision	Date	Description
D.0	28 November 2016	Addition of protocol for KingFisher™ Duo Prime Magnetic Particle Processor
C.0	24 July 2014	Addition of important procedural guidelines
B.0	01 July 2014	Correction of the kit name
A.0	29 May 2014	New document

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