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MagMAX™ DNA Multi-Sample Ultra Kit

High-throughput isolation of PCR-ready DNA from saliva

Catalog Number A25597 and A25598

Pub. No. MAN0010928 **Rev.** A.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 **www.lifetechnologies.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.

This protocol describes isolation of DNA from saliva, optimized for use with the MagMAX™ Express-96 Deep Well Magnetic Particle Processor or with the KingFisher™ Flex Magnetic Particle Processor (96-well deep well setting). The typical DNA yield obtained from 150 µL of saliva is 2–12 µg at a concentration suitable for OpenArray® analysis.

Kit contents and storage

Component	Cat. no. A25597 (500 rxns)	Cat. no. A25598 (2500 rxns)	Storage
Proteinase K[1]	4 mL	5 × 4 mL	–15°C to –25°C
PK Buffer	96 mL	5 × 96 mL	15°C to 30°C
Multi-Sample DNA Lysis Buffer	100 mL	5 × 100 mL	
RNase A	2 × 1.25 mL	10 × 1.25 mL	–15°C to –25°C
DNA Binding Beads[1]	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[2]	5 × 80 mL[2]	
Wash Solution 2 Concentrate	162 mL[2]	5 × 162 mL[2]	
DNA Elution Buffer 1	25 mL	5 × 25 mL	
DNA Elution Buffer 2	25 mL	5 × 25 mL	

[1] Proteinase K is also available as Cat. no. A25561, and DNA Binding Beads are also available as Cat. no. A25562.

[2] Final volume; see "Before first use of the kit: prepare Wash Solutions" on page 2.

Materials required but not supplied

Unless otherwise specified, all materials are available from Life Technologies. MLS: major laboratory supplier.

Item	Source
Magnetic particle processor	
MagMAX™ Express-96 Magnetic Particle Processor	Cat. no. 4400077
KingFisher™ Flex Magnetic Particle Processor[1]	Thermo Scientific Cat. no. 5400630
Equipment	
Thermo Scientific™ Compact Digital Microplate Shaker	Cat. no. 11-676-337[2]
Fisher Scientific™ Analog Vortex Mixer	Cat. no. 02-215-365[2]
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
(Optional but recommended) Magnetic Stand-96	Cat. no. AM10027
Plastics and consumables	
MagMAX™ Express-96 Deep Well Plates	Cat. no. 4388476
MagMAX™ Express-96 Standard Plates	Cat. no. 4388475
MagMAX™ Express-96 Deep Well Tip Combs	Cat. no. 4388487
MicroAmp® Clear Adhesive Film	Cat. no. 4306311
RNase-free Microfuge Tubes (2.0 mL)	Cat. no. AM12425
Conical tubes (15 mL)	Cat. no. AM12500
Conical tubes (50 mL)	Cat. no. AM12502
Aerosol-resistant pipette tips	MLS
Reagent reservoirs	MLS
Reagents	
Ethanol, 200 proof (absolute)	MLS
Isopropanol, 100% (molecular grade or higher)	MLS

[1] See "If needed, download the KingFisher™ Flex program" on page 2.

[2] Available from Fisher Scientific

Sample collection and storage

- Sample collection: Test subjects should thoroughly rinse their mouths with water prior to collection. Collect samples by having the subject discharge saliva into a sterile 15-mL conical tubes.

  Note: We recommend collecting 0.5–1 mL of saliva.
- *(Optional)* Sample storage: Samples can be stored at room temperature or 4°C overnight.

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For Research Use Only. Not for use in diagnostic procedures.

Important procedural guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- When mixing samples by pipetting up and down, avoid creating bubbles.
- 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 Thermo Scientific™ Compact Digital Microplate Shaker, verify that:
  - The plate fits securely on your titer plate shaker.
  - The recommended speeds are compatible with your titer plate shaker. Ideal speeds should 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.

If needed, download the KingFisher™ Flex program

The program required for this protocol is not pre-installed on the KingFisher™ Flex Magnetic Particle Processor.

1. On the MagMAX™ DNA Multi-Sample Ultra Kit web page, scroll down to the **Product Literature** section.
2. Right-click **A25597_Blood_Buccal** and select **Save as Target** to download to your computer.
3. Refer to *Thermo Scientific™ KingFisher™ Flex User Manual* (Cat. no. N07669) and *BindIt™ Software User Manual* (Cat. no. N07974) for instructions for installing the program on the instrument.

Before first use of the kit: prepare Wash Solutions

- 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: prepare DNA Binding Bead Mix

Vortex DNA Binding Beads thoroughly, then combine with Nuclease-free Water according to the following table.

Component	Volume per well	Volume per plate
DNA Binding Beads	16 µL	1.6 mL
Nuclease-free Water	4 µL	0.4 mL
Total DNA Binding Bead Mix	20 µL	2 mL

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

Perform DNA extraction and elution

1 Lyse the samples with Proteinase K, RNase A, and Multi-Sample DNA Lysis Buffer

a. Prepare sufficient PK Mix according to the following table. Invert PK Mix several times to thoroughly mix components.

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

Component	Volume per well	Volume per plate
Proteinase K	0.8 µL	80 µL
PK Buffer	4.2 µL	420 µL
Total PK Mix	5 µL	500 µL

b. Vortex samples, then transfer 150 µL of samples to the wells of a MagMAX™ Express-96 Deep Well Plate (PK Plate).
c. Add 5 µL of PK Mix to each sample.
d. Seal the plate with a MicroAmp® Clear Adhesive Film and shake for 5 minutes at speed 8.
e. Add 75 µL of Multi-Sample DNA Lysis Buffer to each sample.
f. Seal the plate with the same Adhesive Film and shake for 5 minutes at speed 8.
g. Add 5 µL of RNase A to each sample.
h. Seal the plate with the same Adhesive Film and shake for 10 minutes at speed 8.

2 Set up the processing plates

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

Table 1 Processing plates

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 MagMAX™ Express-96 Deep Well Tip Comb in a MagMAX™ Express-96 Deep Well 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.

3 Add isopropanol and DNA Binding Bead Mix

a. Add 180 µL of isopropanol to each sample.
b. Seal the plate with the Adhesive Film and shake for 5 minutes at speed 8.
c. Vortex the DNA Binding Bead Mix at moderate speed to ensure thorough mixing.
d. Add 20 µL of DNA Binding Bead Mix to each sample, and proceed immediately to the next step.

4 Wash the beads and elute the DNA

a. Select the program on the instrument.
   - **4413021 DW blood** on MagMAX™ Express-96 Magnetic Particle Processor
   - **A25597_Blood_Buccal** on KingFisher™ Flex Magnetic Particle Processor

b. Start the run and load the prepared processing plates in their positions when prompted by the instrument (see Table 1).
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.
   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 MicroAmp® Clear Adhesive Film.
   - If precipitated DNA is visible, pipet up and down 5–10 times before sealing the plate, to ensure complete resuspension.
   - *(Optional)* Eluates can be transferred to a storage plate after collection.
   - If excess bead residue is seen 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.

Revision history

Revision	Date	Description
A.0	October 2014	New document

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