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MagMAX™ DNA Multi-Sample Ultra Kit

High-throughput isolation of PCR-ready DNA from blood cards

Catalog Number A25597 and A25598

Pub. No. MAN0010816 **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 mammalian whole blood spotted onto blood storage cards, 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 3-mm^2 sections of card spotted with 40 µL of blood is 50–200 ng at a 0.5–2 ng/µL concentration.

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™ Titer Plate Shaker	Cat. no. 14-271-9[2]
One of the following incubators, or an equivalent incubator with slatted shelves and thermometer:	
Economy Lab Incubator	Cat. no. S50441A[2]
VWR® Digital Mini Incubator	VWR Cat. no. 10055-006
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
Scalpel or hole puncher	MLS
Forceps	MLS
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
Whatman® FTA® Elute Cards, or equivalent	MLS
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
NaCl (5M)	Cat. no. AM9760G
EDTA (0.5M), pH 8.0	Cat. no. AM9260G

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

[2] Available from Fisher Scientific

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A Thermo Fisher Scientific Brand

Sample collection and storage

1. Collect 40 µL of blood samples onto Whatman® FTA® Elute Cards using one of the following methods.

   Note: A different collection volume might be needed for other types of blood cards.

   - **Finger stick:** Collect samples directly on the blood cards.
   - **Venipuncture:** Collect samples in EDTA or sodium citrate anticoagulant tubes, then transfer to blood cards.

     Note: Heparin is not recommended as an anti-coagulant since it can cause inhibition of PCR reactions.
2. Dry the cards at least 4 hours or according to the manufacturer's instructions; lay flat and protect from moisture.
3. Process samples shortly after they are completely dry, or store the cards in a dry place at room temperature.

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.
- Use sterile scalpels or hole punchers, and sterile forceps when preparing samples. Dip them in 70% ethanol between each sample to prevent cross-contamination.
- 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™ Titer Plate 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

1. 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.
2. Prepare High Salt Wash Solution as indicated in the following table, mix, and store at room temperature

Component	Volume	Final concentration
5 M NaCl	40 mL	2 M
0.5 M EDTA, pH 8.0	4 µL	2 mM
Ethanol	40 mL	40% (v/v)
Water	up to 100 mL	—
Total High Salt Wash Solution	100 mL	—

Perform DNA extraction and elution

1 Digest the samples with Proteinase K

a. Preheat an incubator to 65°C.

b. 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	8 µL	800 µL
PK Buffer	192 µL	19.2 mL
Total PK Mix	200 µL	20 mL

c. Add 200 µL of PK Mix to each sample well of a MagMAX™ Express-96 Deep Well Plate (PK Plate).

d. Cut out 1 or 2 pieces of the blood cards, 2–3 mm^2 each, using a scalpel or a hole puncher, and transfer them to the appropriate wells of the PK Plate using forceps.

IMPORTANT! Ensure that the card pieces are entirely submersed in liquid before starting PK digestion.

e. Seal the plate with a MicroAmp® Clear Adhesive Film and shake the sealed plate for 10 minutes at speed 8.

f. Incubate overnight at 65°C, then shake the sealed plate for 5 minutes at speed 8.

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.

2 Set up the processing plates

Just before the 65°C incubation is complete, set up the Wash, Elution, and Tip Comb Plates outside the instrument as described in the following table.

2 Set up the processing plates *(continued)*

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	High Salt Wash Solution	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 Multi-Sample DNA Lysis Buffer, Bead/ RNase Mix, and isopropanol

a. (Optional) If condensation is present at the end of the agitation, centrifuge the plate for 1–2 minutes at 1500 × *g*.
b. Transfer samples to the wells of a new MagMAX™ Express-96 Deep Well Plate and discard the previous plate.
c. Add 200 µL of Multi-Sample DNA Lysis Buffer to each sample.
d. Seal the plate with the MicroAmp® Clear Adhesive Film and shake for 5 minutes at speed 8.
e. Prepare Bead/RNase Mix according to the following table.

IMPORTANT! Prepare the Bead/RNase Mix up to 20 minutes before use. Prolonged storage at room temperature can reduce its efficiency. Vortex the DNA Binding Beads at moderate speed to form a uniform suspension before pipetting.

Component	Volume per well	Volume per plate
DNA Binding Beads	16 µL	1.6 mL
RNase A	5 µL	500 µL
Nuclease-free Water	19 µL	1.9 mL
Total Bead/RNase Mix	40 µL	4 mL

f. Vortex the Bead/RNase Mix at moderate speed to ensure thorough mixing and add 40 µL to each sample, then use a multi-channel micropipettor to mix by pipetting up and down 5 times.
g. Seal the plate with the MicroAmp® Clear Adhesive Film and shake for 5 minutes at speed 8.
h. Add 240 µL of isopropanol to each sample, and proceed immediately to DNA isolation (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 to their positions when prompted by the instrument (see Table 1).
c. Load the PK plate (containing lysate, isopropanol, and Bead/RNase 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.

4 Wash the beads and elute the DNA *(continued)*

- 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.

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).

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

Revision history

Revision	Date	Description
A.0	September 2014	New document

Limited product warranty

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