MagMAX™ DNA Multi-Sample Ultra Kit

High-throughput isolation of PCR-ready DNA from blood cards - 24-well format

Catalog Numbers A25597 and A25598

Pub. No. MAN0016325 **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 **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.

This protocol describes isolation of DNA from mammalian whole blood spotted onto blood storage cards, specifically optimized for use with the KingFisher™ Flex Magnetic Particle Processor (24-well deep well setting). Reagents provided in Cat. Nos. A25597 and A25598 are sufficient for 65 and 325 optimized reactions, respectively. 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	Cat. No. A25598	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" 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 1 Required materials and equipment not included with the kit

Item	Source
Magnetic particle processor	
KingFisher™ Flex Magnetic Particle Processor with 24 Deep-Well Head and 24-well heat block	5400640
Equipment	
Thermo Scientific™ Compact Digital Microplate Shaker	Fisher Scientific 11-676-337
Laboratory incubator with slatted shelves, capable of maintaining 65°C	MLS
Analog Vortex Mixer	Fisher Scientific 02-215-365
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
(*Optional but recommended*) 24-Well Magnetic Separator	CS15024
Plates and combs	
KingFisher™ Flex 24 Deep-Well Plates	95040470
KingFisher™ Flex 24 Deep Well Tip Comb and plate	97002610
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

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. Click **A25597_350uL_Blood** to download the program to your computer.
3. See *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.

Sample collection and storage

- Sample collection: 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.

  Dry the cards at least 4 hours or according to the manufacturer's instructions; lay flat and protect from moisture.

- *(Optional)* Sample storage: Dried cards can be stored 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™ Compact Digital Microplate 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.

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.

Perform DNA extraction and elution

1 Digest the samples with Proteinase K

Ensure that the incubator is preheated to 65°C.

a. 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	Volume per plate
Proteinase K	40 µL	1 mL
PK Buffer	960 µL	24 mL
Total PK Mix	**1000 µL**	**25 mL**

b. Add 1000 µL of PK Mix to each sample well of a deep-well plate (PK Plate).

c. Cut out 1 or 2 pieces of the blood cards, 2–5 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.

d. Seal the plate with a clear adhesive film, then shake the sealed plate at 550 rpm for 5 minutes.

e. Incubate overnight minutes at 65°C, then seal the plate with a clear adhesive film, then shake the sealed plate at 550 rpm for 5 minutes..

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

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

Table 2 Processing plates

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well
Wash Plate 1	2	Deep Well	Wash Solution 1	1 mL
Wash Plate 2	3	Deep Well	Wash Solution 2	1 mL
Wash Plate 3	4	Deep Well	Wash Solution 2	1 mL
Elution Plate[2]	5	Deep Well	DNA Elution Buffer 1	200 µL
Tip Comb	6	Deep Well	Use the pre-assembled KingFisher™ Flex 24 Deep Well Tip Comb and 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.

3 Add Multi-Sample DNA Lysis Buffer, Bead/ RNase Mix, and isopropanol

a. Centrifuge the plate at 1,500 × *g* for 1–2 minutes, then transfer the supernatant to a new plate leaving the blood card material behind.

b. Add 1 mL of Multi-Sample DNA Lysis Buffer to each sample.

c. Seal the PK plate with a clear adhesive film, then shake at 550 rpm for 5 minutes.

3 Add Multi-Sample DNA Lysis Buffer, Bead/ RNase Mix, and isopropanol *(continued)*

d. Prepare Bead/RNase A Mix according to the following table.

IMPORTANT! Prepare the Bead/RNase A Mix no more than 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 preparing the Bead/RNase A Mix.

Component	Volume per well	Volume per plate
DNA Binding Beads	80 µL	2000 µL
RNase A	25 µL	625 µL
Total Bead/RNase A Mix	**105 µL**	**2625 µL**

e. Vortex the Bead/RNase A Mix at moderate speed to ensure thorough mixing, then add 105 µL to each sample and pipet up and down 5 times using a micropipettor.

Note: If you see that the beads in the Bead/RNase A Mix are settling, vortex the mix again briefly before continuing to pipette.

f. Seal the PK plate with the clear adhesive film, then shake at 550 rpm for 5 minutes.

g. Add 1.1 mL of isopropanol to each sample, then proceed immediately to DNA isolation (next section).

4 Process samples on the instrument

a. Select the program **A25597_350uL_Blood** on the instrument.

b. Start the run, remove the temporary paraffin plate seals (if present), and load the prepared processing plates to their positions when prompted by the instrument (see Table 2).

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

d. When prompted by the instrument (approximately 29 minutes after initial start):

1. Remove the Elution Plate from the instrument.
2. Add 200 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 34 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 24-Well Magnetic Separator for up to 5 minutes 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 2–8°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.

Limited product warranty

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Revision	Date	Description
A.0	28 November 2016	New document

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