

MagMAX™ DNA Multi-Sample Ultra Kit

High-throughput isolation of PCR-ready DNA from large volumes (0.5 or 1 mL) of whole blood

Catalog Numbers A25597 and A25598

Pub. No. MAN0015998 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 a modified workflow for isolation of DNA from mammalian whole blood samples, specifically optimized for use with large volumes of whole blood on the KingFisher™ Flex Magnetic Particle Processor (24-well deep well setting). Reagents provided in Cat. Nos. A25597 and A25598 plus extra bottles of each Wash Solution Concentrate (purchased separately) are sufficient for 65 and 325 optimized reactions, respectively. The typical DNA yield obtained from 1 mL of whole blood is 30-66 µg (depending on pre-analytical variables and the donor's health) at a concentration of 50–110 ng/µL (by OD₂₆₀), which is suitable for OpenArray™ analysis.

Kit contents and storage

Component	Cat. No. A25597 ^[1] (500 rxns)	Cat. No. A25598 ^[2] (2500 rxns)	Storage
Proteinase K ^[3]	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 ^[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 ^[4]	5 × 80 mL ^[4]	
Wash Solution 2 Concentrate	162 mL ^[4]	5 × 162 mL ^[4]	
DNA Elution Buffer 1	25 mL	5 × 25 mL	
DNA Elution Buffer 2	25 mL	5 × 25 mL	

^[1] One bottle of MagMAX™ Wash Solution 1 Concentrate [Cat. No. AM8504] and 2 bottles of MagMAX™ Wash Solution 2 Concentrate [Cat. No. AM8640] are also needed.

^[2] 5 bottles of MagMAX™ Wash Solution 1 Concentrate [Cat. No. AM8504] and 7 bottles of MagMAX™ Wash Solution 2 Concentrate [Cat. No. AM8640] are also needed.

^[3] Proteinase K is also available as Cat. No. A25561 and DNA Binding Beads are also available as Cat. No. A25562.

^[4] 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 (www.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
<i>(Optional but recommended)</i> 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
<i>(Optional)</i> Paraffin film	MLS
Reagents	
MagMAX™ Wash Solution 1 Concentrate	AM8504
MagMAX™ Wash Solution 2 Concentrate	AM8640
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_Large_Volume_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 blood samples using proper venipuncture collection and handling procedures in EDTA or sodium citrate anticoagulant tubes. Invert the tube to ensure thorough mixing.
Note: Heparin is not recommended as an anti-coagulant since it can cause inhibition of PCR.
- (Optional) Sample storage: Store samples between -20°C and -80°C . We recommend storing samples in smaller volumes to prevent multiple freeze/thaw cycles.

Procedural guidelines

- If the whole blood is frozen prior to use, thaw the sample at $25\text{--}37^{\circ}\text{C}$ in a water bath until it is completely liquid, and place on ice until needed.
- Perform all steps at room temperature ($20\text{--}25^{\circ}\text{C}$) 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 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.

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.
 - Add 70 mL of isopropanol to MagMAX™ Wash Solution 1 Concentrate (Cat. No. AM8504), mix, and store at room temperature.
 - Add 160 mL of ethanol to MagMAX™ Wash Solution 2 Concentrate (Cat. No. AM8640), 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 .

- 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	
	0.5-mL blood sample	1-mL blood sample	0.5-mL blood sample	1-mL blood sample
Proteinase K	50 μL	50 μL	1.25 mL	1.25 mL
PK Buffer	1200 μL	700 μL	30 mL	17.5 mL
Total PK Mix	1250 μL	750 μL	31.25 mL	18.75 mL

- Add PK Mix to each sample well of a deep-well plate (PK Plate) according to the following table.

Sample size	PK Mix volume
0.5 mL	1250 μL
1 mL	750 μL

- Transfer 0.5 mL or 1 mL of whole blood to the appropriate well containing PK Mix.

IMPORTANT! Invert the tube containing the blood sample before pipetting to ensure homogenous mixing.

- Seal the plate with a clear adhesive film, then shake the sealed plate for 5 minutes at 550 rpm.
- Incubate for 10 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.

- 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	4 mL
Wash Plate 2	3	Deep Well	Wash Solution 2	4 mL
Wash Plate 3	4	Deep Well	Wash Solution 2	2 mL
Elution Plate ^[2]	5	Deep Well	DNA Elution Buffer 1	300 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 Bead/RNase A Mix, Multi-Sample DNA Lysis Buffer, and isopropanol

- a. Prepare sufficient 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	115 µL	2.9 mL
RNase A	35 µL	882 µL
Total Bead/RNase A Mix	150 µL	3.78 mL

- b. (Optional) If condensation is present at the end of the incubation, centrifuge the plate for 1–2 minutes at 1,500 × g.
- c. Vortex the Bead/RNase A Mix at moderate speed to ensure thorough mixing, then add 150 µL to each sample.
- Note:** If you see that the beads in the Bead/RNase A Mix are settling, vortex the mix again briefly before continuing to pipette.
- d. Seal the PK plate with the clear adhesive film, then shake for 5 minutes at 550 rpm.
- e. Add 1.4 mL of Multi-Sample DNA Lysis Buffer to each sample and pipet up and down 5 times using a micropipettor.
- f. Add 1.7 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_Large_Volume_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 “Set up the processing plates” on page 3).
- 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 31 minutes after initial start):
1. Remove the Elution Plate from the instrument.
 2. Add 300 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 38 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 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	12 August 2016	New document

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