MagMAX™ DNA Multi-Sample Ultra Kit

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

Catalog Numbers A25597 and A25598

Pub. No. MAN0010294 **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.

This protocol describes isolation of DNA from mammalian whole blood samples, 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 50 µL of whole blood is 1.5–4 µg at a concentration 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] 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] 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.

Table 3 Additional materials and equipment required for processing whole blood samples

Item	Source
Laboratory incubator with slatted shelves, capable of maintaining 65°C	MLS
(*Optional*) Proteinase K, 500 reactions (4 mL)	A25561
(*Optional*) DNA Binding Beads, 500 reactions (8 mL)	A25562

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.

Guidelines for whole blood preparation

- If the whole blood is frozen prior to use, thaw the sample at 25–37°C in a water bath until it is completely liquid, then place on ice until needed.
- 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 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.
- If the DNA yield is lower than expected, extend the Proteinase K digestion to 45 minutes.

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.

Digest the samples with Proteinase K

Ensure that the incubator is preheated to 65°C.

1. 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**

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

   If you are using the KingFisher™ Duo Prime Magnetic Particle Processor, make sure to add the PK Mix to the wells of Row H.
3. Transfer 50 µL of whole blood to the appropriate well containing PK Mix.

   IMPORTANT! Invert the tube containing the blood sample before pipetting to ensure homogenous mixing.
4. Seal the plate with a clear adhesive film, then shake the sealed plate at 900–950 rpm for 5 minutes.
5. 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 Bead/RNase A 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 PK plate with a clear adhesive film, then shake at 900–950 rpm for 5 minutes.

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
DNA Binding Beads	16 µL
RNase A	5 µL
Nuclease-free Water	19 µL
Total Bead/RNase A Mix	**40 µL**

e. Vortex the Bead/RNase A Mix at moderate speed to ensure thorough mixing, then add 40 µL to each sample and pipet up and down 5 times using a multi-channel micropipettor.

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 900–950 rpm for 5 minutes.

g. Add 240 µL of isopropanol to each sample, then proceed immediately to the next section.

3 Process samples on the instrument

a. Select the program on the instrument.

- KingFisher™ Flex Magnetic Particle Processor: **A25597_Blood_Buccal**
- MagMAX™ Express-96 Magnetic Particle Processor: **4413021 DW blood**

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.
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.

3 Process samples on the instrument *(continued)*

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 Bead/RNase A 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 PK plate with a clear adhesive film, then shake at 900–950 rpm for 5 minutes.

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
DNA Binding Beads	16 µL
RNase A	5 µL
Nuclease-free Water	19 µL
Total Bead/RNase A Mix	**40 µL**

e. Vortex the Bead/RNase A Mix at moderate speed to ensure thorough mixing, then add 40 µL to each sample and pipet up and down 5 times using a multi-channel micropipettor.

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 900–950 rpm for 5 minutes.

g. Add 240 µL of isopropanol to each sample, then proceed immediately to the next section.

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.
- If precipitated DNA is visible, pipet up and down 5–10 times before sealing the plate, to ensure complete resuspension.

3 Process samples on the instrument *(continued)*

- 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.

Limited product warranty

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Revision history: Pub. No. MAN0010294

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

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