MagMAX™ DNA Multi-Sample Ultra Kit

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

Catalog Number A25597 and A25598

Pub. No. MAN0010818 **Rev.** B.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 blood leukocytes, optimized for use with the KingFisher™ Flex Magnetic Particle Processor (96-well deep well setting). The typical DNA yield obtained from leukocytes from 200 µL of whole blood is 3–9 µg at a concentration of 30–90 ng/µL, which is suitable for OpenArray™ analysis. The protocol can also be used on the MagMAX™ Express-96 Deep Well Magnetic Particle Processor, with a slightly lower yield.

Contents and storage

Contents	Cat. no. A25597 (500 rxns)	Cat. no. A25598 (2500 rxns)	Storage
Proteinase K[1]	4 mL	5 × 4 mL	–25°C to –15°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	–25°C to –15°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 (**www.fisherscientific.com**) or other major laboratory supplier.

Table 1 Required materials not included with the kit

Item	Source
One of the following instruments	
(Recommended) KingFisher™ Flex Magnetic Particle Processor[1]	Cat. no. 5400630
MagMAX™ Express-96 Magnetic Particle Processor	—[2]
Equipment	
Plate shaker capable of shaking plates at a minimum of 900 rpm	MLS
Analog Vortex Mixer	Fisher Scientific 02-215-365
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
(*Optional but recommended*) Magnetic Stand-96	Cat. no. AM10027
Plates and combs	
Deep Well Plates, one of the following:	
KingFisher™ Flex Microtiter Deepwell 96 Plate, Sterile	Cat. no. 95040460
MagMAX™ Express-96 Deep Well Plates	Cat. no. 4388476
Standard Well Plates, one of the following:	
KingFisher™ 96 KF Microplate	Cat. no. 97002540
MagMAX™ Express-96 Standard Plates	Cat. no. 4388475
Tip Combs, one of the following:	
KingFisher™ 96 Tip Comb for DW Magnets	Cat. no. 97002534
MagMAX™ Express-96 Deep Well Tip Combs	Cat. no. 4388487
Other consumables	
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
(*Optional*) Paraffin film	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] Not available for sale.

Table 2 Additional materials required for blood leukocytes

Item	Source
Centrifuge capable of spinning deep well plates at 1700 × *g*	MLS
Laboratory incubator with slatted shelves capable of reaching 65°C	MLS
Ammonium Chloride (A.C.S. grade or higher)	MLS
EDTA (0.5M), pH 8.0	Cat. no. AM9260G

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 reactions.
- *(Optional)* Sample storage: Samples can be stored frozen 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 25–37°C in a water bath until it is completely liquid, and 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.
- Perform incubation in ice by covering the plate in ice up to the sides without covering the top of the wells.
- 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 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_Leukocytes** 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 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.
- Prepare 10X Red Blood Cell (RBC) Buffer as indicated in the following table, mix, and store at 2–8°C.

Component	Quantity	Final concentration
Ammonium chloride	9 g	1.68 M
0.5 M EDTA, pH 8.0	400 µL	2 mM
Water	up to 100 mL	—
Total 10X RBC Buffer	100 mL	—

Before each use of the kit

- Preheat the incubator to 65°C.
- Prepare fresh 1X RBC Buffer as indicated in the following table, mix, and store on ice until use.

Component	Volume per well	Volume per plate
10X RBC Buffer	60 µL	6 mL
Water	540 µL	54 mL
Total 1X RBC Buffer	600 µL	60 mL

Perform DNA extraction and elution

1 Lyse the red blood cells and pellet the leukocytes

a. Invert the collection tube 5 times to ensure thorough mixing of the sample.
b. Transfer 200 µL of whole blood to each sample well.
c. Add 600 µL of ice-cold 1X RBC Buffer to each sample and mix by pipetting up and down 10 times with a multi-channel micropipettor.
d. Seal the plate with a MicroAmp™ Clear Adhesive Film.
e. Submerge the plate in ice that is halfway up the side of the plate, then incubate 15 minutes. Mix briefly twice during the incubation by shaking the plate for 15 seconds at 900–950 rpm.

   Note: The cloudy suspension becomes translucent during incubation, indicating lysis of the erythrocytes.
f. Centrifuge at ≥1700 × *g* for 15 minutes.

1 Lyse the red blood cells and pellet the leukocytes *(continued)*

g. Carefully remove and discard the supernatant (~700 µL) without disturbing the leukocyte pellet.

Note: Up to 100 µL of supernatant can be left in the well to avoid loss of sample. When removing the supernatant, aspirate from the side of the well, not the center.

h. Seal the plate with the MicroAmp™ Clear Adhesive Film, then place on ice.

2 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. 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	5.0 µL	500 µL

b. Add 5 µL of PK Mix to each sample well and mix by pipetting up and down 5 times with a multi-channel micropipettor.

c. Seal the plate with a MicroAmp™ Clear Adhesive Film and shake the sealed plate for 5 minutes at 900–950 rpm.

d. Incubate for 15 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.

During the incubation at 65°C, follow "Set up the processing plates" on page 3.

Proceed immediately to "Add Multi-Sample DNA Lysis Buffer, Bead/RNase A Mix, and isopropanol" on page 3.

3 Set up the processing plates

a. During the incubation at 65°C, set up the Wash, Elution, and Tip Comb Plates outside the instrument as described in the following table.

Table 3 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.

b. (*Optional*) To prevent evaporation and contamination, cover the prepared processing plates with paraffin film until they are loaded into the instrument.

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

a. Add 400 µL of Multi-Sample DNA Lysis Buffer to each sample and mix by pipetting up and down 5 times with a multi-channel micropipettor.

b. Seal the plate with the MicroAmp™ Clear Adhesive Film and shake for 5 minutes at 900–950 rpm.

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

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

IMPORTANT! Prepare the Bead/RNase A 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 A Mix	40 µL	4 mL

d. Vortex the Bead/RNase A 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.

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

e. Seal the plate with the MicroAmp™ Clear Adhesive Film and shake for 5 minutes at 900–950 rpm.

f. Add 240 µL of isopropanol to each sample, briefly shake for 30 seconds at 900–950 rpm, then proceed immediately to DNA isolation (next step).

5 Process samples on the instrument

a. Select the program **A25597_Leukocytes** on the instrument.

If you are running your samples on the MagMAX™ Express-96 Magnetic Particle Processor, use the program **4413021_DW_blood**.

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 3).

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

d. When prompted by the instrument (approximately 23 minutes after initial run):

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 27 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
B.0	December 2015	Addition of a Proteinase K digestion step
A.0	September 2014	New document

Limited product warranty

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