MagMAX™ DNA Multi-Sample Ultra 2.0 Kit

High throughput isolation of DNA from blood cards

Catalog Number A36570

Pub. No. MAN0017456 **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 description

The Applied Biosystems™ MagMAX™ DNA Multi-Sample Ultra 2.0 Kit is developed for scalable, rapid purification of high-quality DNA from a variety of sample matrices. DNA purified with this kit can be used in a broad range of molecular biology downstream applications, such as sequencing, genotyping, and qPCR. This protocol guides through automated isolation of DNA from mammalian whole blood spotted onto blood cards, optimized for use with Whatman™ 903 Cards and Whatman™ FTA™ Classic Cards, using the KingFisher™ Flex and the KingFisher™ Duo Prime.

Contents and storage

Reagents provided in the kit are sufficient for 100 reactions.

Table 1 MagMAX™ DNA Multi-Sample Ultra 2.0 Kit (Cat. No. A36570)

Component	Quantity	Storage
Enhancer Solution	4.5 mL	15–30°C
Proteinase K	4.5 mL	
Binding Solution	45 mL	
DNA Binding Beads	4.5 mL	
Wash I Solution	110 mL	
Elution Solution	12 mL	

For 1000 reaction volume use Cat. No. A36578 (Proteinase K), A36579 (DNA Binding Beads), A36580 (Wash I Solution), A36581 (Lysis/binding Solution), A36582 (Elution Solution), and A36583 (Enhancer Solution).

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Magnetic particle processor (one of the following):	
KingFisher™ Duo Prime Magnetic Particle Processor	5400110
KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head	5400630
Equipment	
One of the following hole punchers:	
Whatman™ Uni-Core Punch	09-924-589
Miltex™ Biopsy Punch	MLS
Incubator with metal racks	MLS
Compact Digital Microplate Shaker	88880023
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Consumables	
Deep-well plates:	
KingFisher™ Deepwell 96 Plate	95040450
96-well standard plates (for use with KingFisher™ Flex only; tip comb placement and eluate storage):	
KingFisher™ 96 KF microplate	97002540
Tip comb, compatible with the magnetic particle processor used:	
12-tip comb, for Microtiter 96 Deepwell plate	97003500
KingFisher™ 96 tip comb for DW magnets, KingFisher™ Flex protocol only	97002534

Item	Source
Materials	
MicroAmp™ Clear Adhesive Film	4306311
One of the following blood cards:	
Whatman™ 903 Protein Saver Card	05-715-121
Whatman™ 903 Neonatal Screening Cards	09-800-961
Whatman™ FTA Classic Cards	09-923-341 or 09-923-340 fisherscientific.com
Reagents	
Ethanol, 96–100% (molecular biology grade)	MLS
Nuclease-free Water	AM9932
Phosphate Buffered Saline (PBS, pH 7.4)	10010023

General guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Precipitates and high viscosity can occur if Enhancer Solution and Binding Solution are stored when room temperature is too cold. If this occurs, warm them at 37°C and gently mix to dissolve precipitates and reduce viscosity. Avoid creating bubbles.
- Yellowing of the Binding and Wash I Solution is normal and will not affect buffer performance
- 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 using a plate shaker other than the recommended shaker, verify that:
  a. The plate fits securely on the plate shaker.
  b. The recommended speeds are compatible with the 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 10% overage.
- *(Optional)* To prevent evaporation and contamination, cover the prepared processing plates with paraffin film or MicroAmp™ Clear Adhesive Film until they are loaded into the instrument.

Guidelines for Proteinase K digestion

- Do not pre-mix the Enhancer Solution and Proteinase K.
- Do not change the order of pipetting.

Guidelines for DNA Binding Bead Mix

- Vortex the DNA Binding Beads thoroughly, combine them with the Binding Solution in a nuclease-free tube, then invert the tube until homogeneous. This mixture can be stored for up to 1 day before aliquoting into the plates.
- Ensure that the beads stay fully mixed within the solution during pipetting.
- Avoid creating bubbles during mixing and aliquoting.

Sample collection and storage

1. Collect blood samples onto Whatman™ 903 Cards using a method from the following table.

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

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

   IMPORTANT! Heparin is not recommended as an anti-coagulant as it can cause inhibition of PCR reactions.

2. Dry the cards at room temperature for at least 3 hours, or according to the manufacturers' instructions.

   Lay cards on a flat surface and allow to completely air dry.

3. Process samples shortly after they are completely dry or, store the cards at room temperature with a desiccant packet or in a humidity-controlled, cool, and dry environment.

Before first use of the kit

Prepare Wash II Solution: Make 80% ethanol from 100% absolute ethanol and Nuclease-Free Water.

Before each use of the kit

Vortex DNA Binding Beads to fully resuspend the beads before each use.

Prepare samples and digest with Proteinase K

1 **Prepare samples and digest with Proteinase K**

Preheat an incubator to 65°C.

a. Cut out the blood cards using a Whatman™ Uni-Core punch or a Miltex™ biopsy punch.

b. Prepare 2-, 3-, or 4-mm sized discs, using 1 or 2 discs per reaction, then transfer them to the appropriate wells of the Proteinase K 96-well DW Plate using forceps.

1 Prepare samples and digest with Proteinase K *(continued)*

c. Prepare sufficient Proteinase K Mix according to the following table, then gently invert or pipet up and down several times to thoroughly mix components.

Component [1]	Volume per well	Volume per plate (96 samples)
Enhancer Solution	40 µL	4.22 mL
Nuclease-free Water	400 µL	42.24 mL
Proteinase K	40 µL	4.22 mL
Total volume	**480 µL**	**50.68 mL**

[1] Pipet the components in the order they are listed in the table.

IMPORTANT! Only make enough Mix for immediate use. Mix is not stable for prolonged periods and will result in a reduction of DNA yield.

d. Add 480 µL of the Proteinase K Mix to each well containing a punched disc.

Be careful to avoid touching the pipette tip to the disc when pipetting the Proteinase K Mix into the sample wells.

IMPORTANT! Ensure that the card pieces are entirely covered in liquid before starting Proteinase K digestion.

e. Seal the plate with the clear adhesive film, then shake the sealed plate at **900 rpm for 5 minutes**.

f. Take the plate off the plate shaker, then immediately incubate at **65°C for 30 minutes**.

Note: For Whatman™ FTA™ Classic Cards, we recommend overnight incubation for optimal yield.

IMPORTANT! Arrange plate in the incubator to allow adequate flow around the plate wells to ensure that samples quickly reach and maintain the incubation temperature.

g. Once incubation is complete, shake the sealed plate at **900 rpm for 5 minutes**.

h. (*Optional*) If condensation is present at the end of the agitation, centrifuge the plate for 1 minute at 1500 × *g*.

During the incubation, proceed with instrument and plate set up:

- For automated purification using KingFisher™ Flex Magnetic Particle Processor, proceed to "Perform DNA purification using KingFisher™ Flex" on page 3.
- For automated purification using KingFisher™ Duo Prime Magnetic Particle Processor, proceed to "Perform DNA purification using KingFisher™ Duo Prime" on page 5.

Perform DNA purification using KingFisher™ Flex

1 Set up the instrument

a. Ensure that the instrument is set up with the proper magnetic head and the proper heat block, as indicated in the following table.

Component	Type
Magnetic head	96 deep-well magnetic head
Heat block	96 well standard heat block

IMPORTANT! Failure to use the proper magnetic head and heat block will result in lower yields.

b. Ensure that the proper program (**MagMAX_Ultra2_Direct_FLEX**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plates

During the incubation at 65°C, set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table.

Plate ID	Plate position	Plate type	Reagent	Volume per well
Wash I Solution Plate	2	Deep Well	Wash I Solution	1000 µL
Wash II Solution Plate 1	3	Deep Well	Wash II Solution	1000 µL
Wash II Solution Plate 2	4	Deep Well	Wash II Solution	500 µL
Elution Plate	5	Standard	Elution Solution	50 µL
Tip Comb	6	Place a 96 Deep-well Tip Comb in a Standard Plate		

Note: The plates will be loaded onto the instrument immediately after the Sample Plate has been prepared.

3 Bind the gDNA

a. Prepare the DNA Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate (96 samples)
Binding Solution	400 µL	42.24 mL
DNA Binding Beads	40 µL	4.22 mL
Total volume	**440 µL**	**46.46 mL**

b. At the end of Proteinase K digestion, transfer the lysates to the corresponding wells of a new deep-well plate (this will be called the Sample Plate), then discard the blood card discs.

To remove the blood card discs from the lysate	Procedure
Transfer all the lysate volume to a new plate. This will vary by the number of discs used.	1. Set a multi-channel pipettor to 480 µL and transfer one row at a time. 2. Ensure each well contains 350–480 µL after transfer.

Note: Some lysate volume will remain absorbed to the blood card discs after Proteinase K treatment. Increasing the number of discs used will result in increased sample loss.

c. Add 440 µL of DNA Binding Bead Mix to each sample.

Note: Remix Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. Mixture is viscous, pipet slowly to ensure that the correct amount is added.

IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

d. Immediately process the plates on the KingFisher™ Flex Magnetic Particle Processor 96DW.

4 Wash and elute the gDNA

a. Select the program **MagMAX_Ultra2_Direct_FLEX** on the instrument.

b. Start the run, and load the prepared plates into position when prompted by the instrument.

c. At the end of the run, immediately remove the plates from the instrument.

d. Transfer the eluate to the final tube/plate of choice for final storage.

Note: If preferred, the elution plate may be used for final storage of the DNA.

The purified DNA is ready for immediate use. Alternatively, store the plate at –20°C for long-term storage.

Perform DNA purification using KingFisher™ Duo Prime

1 Set up the instrument

a. Ensure that the instrument is set up for processing with the proper magnetic head (12 pin) and heat block for your application.

b. Ensure that the proper program (**MagMAX_Ultra2_Direct_DUO**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plate

Add processing reagents to the wells of the 96-well plate according to the following table.

Row ID	Plate Row	Reagent	Volume per well
Elution Solution	A	Elution Solution	50 µL
Tip Comb	B	Tip Comb	N/A
—	C	Empty	
Wash II Solution	D	Wash II Solution	500 µL
Wash II Solution	E	Wash II Solution	1000 µL
Wash I Solution	F	Wash I Solution	1000 µL
—	G	Empty	
Sample	H	Sample[1]	Varies

[1] See "Bind the gDNA" on page 5.

Note: The plate will be loaded onto the instrument immediately after the Sample Row has been prepared.

3 Bind the gDNA

a. Prepare the DNA Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate (12 samples)
Binding Buffer	400 µL	5.28 mL
DNA Binding Beads	40 µL	528 µL
Total volume	**440 µL**	**5.81 mL**

b. At the end of Proteinase K digestion, transfer the lysates to the corresponding wells (Row H) of the previously prepared processing plate, then discard the blood card discs.

To remove the blood card discs from the lysate	Procedure
Transfer all the lysate volume to a new plate. This will vary by the number of discs used.	1. Set a multi-channel pipettor to 480 µL and transfer one row at a time. 2. Ensure each well contains 350–480 µL after transfer.

Note: Some lysate volume will remain absorbed to the blood card discs after Proteinase K treatment. Increasing the number of discs used will result in increased sample loss.

c. Add 440 µL of DNA Binding Bead Mix to each sample (Row H).

Note: Remix Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. Mixture is viscous, pipet slowly to ensure correct amount is added.

IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

d. Immediately process the plate on the KingFisher™ Duo Prime Magnetic Particle Processor.

4 Wash and elute the gDNA

a. Select the program **MagMAX_Ultra2_Direct_DUO** on the instrument.

b. Start the run, and load the prepared plate into position when prompted by the instrument.

c. At the end of the run, immediately remove the plate from the instrument.

d. Transfer the eluate to the final tube/plate of choice for final storage.

The purified DNA is ready for immediate use. Alternatively, store the samples at –20°C for long-term storage.

Quantitation

To most accurately quantitate gDNA samples isolated from blood cards, it is recommended to quantitate using the Qubit™ dsDNA HS (High Sensitivity) Assay Kit (Cat. No. Q32851). Another acceptable method is quantitation utilizing qPCR and the Applied Biosystems™ TaqMan® RNase P Detection Reagents Kit (Cat. No. 4316831).

or TaqMan® Copy Number Reference Assay, human, RNase P (Cat. No. 4403326) 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 Application Note* (Pub. No. 4371090).

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

Revision history: Pub. No. MAN0017456

Revision	Date	Description
B.0	15 March 2019	Updated manufacturing address to Vilnius. Added note to address yellowing buffers and viscosity concerns.
A.0	30 March 2018	New document