Automated Protocols for Cell-Free Total Nucleic Acid Isolation Suitable for Non-NGS Applications

for use with: MagMAX™ Cell-Free Total Nucleic Acid Isolation Kit

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Note: For safety and biohazard guidelines, see the "Safety" appendix in the *MagMAX™ Cell-Free Total Nucleic Acid Isolation Kit User Guide* (Pub. no. MAN0017274). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This guide describes alternative isolation protocols for 2– or 4–mL plasma samples on the KingFisher™ Flex and KingFisher™ Duo Prime automated platforms. These protocols elute the final sample directly in the 24–deep-well plate in a final elution volume of 30 µL, with no rebinding step required. Samples processed with these modified protocols are suitable for a broad range of applications, including genotyping, qPCR, and dPCR.

IMPORTANT! These alternative protocols are not recommended for downstream analysis with next generation sequencing (NGS) applications. To isolate cfNA suitable for next generation sequencing, or other applications requiring a more concentrated cfNA sample, refer to *MagMAX™ Cell-Free Total Nucleic Acid Isolation Kit User Guide* (Pub. No. MAN0017274).

Product description

The Applied Biosystems™ MagMAX™ Cell-Free Total Nucleic Acid Isolation Kit is designed for isolation of circulating nucleic acid from cell-free human plasma samples. The kit uses Dynabeads™ MyOne™ SILANE technology and extraction chemistry, ensuring reproducible recovery of high-quality cell-free nucleic acid (cfNA), including cell-free DNA, cell-free RNA, and cell-free miRNA.

Two optimized methods are included:

- KingFisher™ Flex Magnetic Particle Processor 24–deep well only
- KingFisher™ Duo Prime Magnetic Particle Processor 24–deep well only

The MagMAX™ Cell-Free Total Nucleic Acid Isolation Kit is optimized for samples that are collected in K_2EDTA and Streck Cell-Free DNA Blood Collection Tubes (BCT).

Contents and storage

Reagents that are provided in the kit are sufficient for 50 reactions from 2 mL of plasma or 25 reactions from 4 mL of plasma.

Table 1 MagMAX™ Cell-Free Total Nucleic Acid Isolation Kit (Cat. No. A36716)

Component	Volume	Storage
MagMAX™ Cell-Free Total Nucleic Acid Lysis/Binding Solution	175 mL	15°C to 25°C
MagMAX™ Cell-Free Total Nucleic Acid Wash Solution Concentrate	66 mL	
MagMAX™ Cell-Free Total Nucleic Acid Elution Solution	21 mL	
MagMAX™ Cell-Free Total Nucleic Acid Magnetic Beads	3.5 mL	2–8°C
MagMAX™ Cell-Free Total Nucleic Acid Proteinase K (20 mg/mL)	1.5 mL	–20°C

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 Materials required for cfNA isolation (all methods)

Item	Source
Instrument	
Magnetic particle processor, one of the following:	
KingFisher™ Duo Prime Magnetic Particle Processor with 6-well magnetic head	5400110
KingFisher™ Flex with 24 deep-well head	5400640
Plates and combs:	
KingFisher™ Flex 24 deep-well plates	95040470 or 95040480
For Flex only: KingFisher™ Flex 24 deep-well tip comb and plate	97002610
For Duo only: KingFisher™ Duo Prime Magnetic Particle Processor 6-tip comb and 24 deep-well plate	97003510
Equipment	
Lab-Line Orbital shaker or similar capable of achieving 1000 rpm	MLS
Fisher Scientific™ Incubating Mini-Shaker with 50-mL conical holder	02-217-753
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Materials	
50-mL conical tubes	4306311
Non-stick, RNase-free, 1.5-mL microfuge tubes	4473979
Reagent reservoirs	MLS
MicroAmp Clear Adhesive Film	4306311
Reagents	
Ethanol, 100% (molecular biology grade)	MLS
Isopropanol, 100% (molecular biology grade)	MLS
Nuclease-Free Water	AM9932

Procedural guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Precipitates can occur if Lysis/Binding Solution and Wash Solution Concentrate are stored when room temperature is too cold. If there are precipitates in these solutions, warm them at 37°C for 1 hour and gently mix to dissolve precipitates. Avoid creating bubbles.
- Vortex the MagMAX™ Cell-Free Total Nucleic Acid Magnetic Beads to resuspend them before use.
- Pipet beads slowly and ensure that they remain in homogeneous suspension while pipetting. Revortexing may be required and low retention pipette tips should be used.
- Use a P100 pipette or larger when handling beads.
- The protocol is compatible with both K_2-EDTA and Streck cfDNA BCTs. K_2-EDTA tubes are the recommended tube for collection of whole blood. Centrifuge the blood to cell-free plasma as soon as possible for best results. If cell-free plasma is frozen, avoid multiple freeze-thaw cycles. Thaw plasma gently and minimize time plasma is held on ice to protect the nucleic acids from degradation.

Before you begin

Before first use of the kit

- Prepare fresh 80% Ethanol using Nuclease-Free Water.
- Prepare Wash Solution 1: Add 34 mL 100% isopropanol to the 66 mL of MagMAX™ Total Nucleic Acid Wash Solution Concentrate.

Before each use of kit

- Bring the MagMAX™ Cell-Free Total Nucleic Acid Magnetic Beads to room temperature and **vortex beads thoroughly**.
- Set shaking incubator to 65°C for Proteinase K digest.

Prepare the Binding Slurry

Preparing the Binding Slurry ensures effective bead mixing on the Kingfisher automated platforms and maximizes cfNA yield. Individual addition of the Binding Slurry components to the Kingfisher plates is not recommended as it decreases cfNA yield. Always prepare fresh and discard any unused portion.

- Prepare **Binding Slurry** according to the following table.

Table 3 Binding Slurry

Binding Slurry Component	Volume of plasma per sample [1]	
	2 mL	4 mL
MagMAX™ Cell-Free Total Nucleic Acid Lysis/Binding Solution	1.5 mL	3 mL
MagMAX™ Cell-Free Total Nucleic Acid Magnetic Beads	60 µL	120 µL
Total volume	**1.56 mL**	**3.12 mL**

[1] Prepare sufficient Binding Slurry for all samples plus 10% overage.

IMPORTANT! Vortex the tube containing the Slurry until all beads are evenly distributed.

Extract cfNA using KingFisher™ Flex

Prepare cell-free plasma

1. Centrifuge the blood sample at 2000 × *g* for 10 minutes at 4°C.
2. Transfer the plasma to a new centrifuge tube, taking care not to disturb the buffy coat layer.
3. Centrifuge the plasma sample at 16,000 × *g* for 10 minutes at 4°C.
4. Transfer the supernatant to a fresh tube for Proteinase K digestion, noting the volume of plasma.

Digest with Proteinase K

1. Set up Proteinase K digestion in a 50-mL conical tube. Combine reagents in the order that is listed according to the following table.

Proteinase K Digest Component	Cell-free plasma volume	
	2 mL	4 mL
MagMAX™ Cell-Free Total Nucleic Acid Proteinase K (20 mg/mL)	30 µL	60 µL
Cell-free plasma	2 mL	4 mL
MagMAX™ Cell-Free Total Nucleic Acid Lysis/Binding Solution	1 mL	2 mL
Total volume	**3.03 mL**	**6.06 mL**

2. Incubate for 30 minutes at 65°C with shaking at 1000 rpm.
3. At the end of Proteinase K incubation, cool the sample on ice for 5 minutes, or until the mix reaches room temperature.

Set up the 24DW processing plates

During Proteinase K incubation, set up the 24DW processing plates outside the instrument according to the following table.

Note: Ensure that the Binding Slurry is prepared according to Table 3 and well vortexed before adding to the plate. Pipet slowly as solution is viscous. It is not necessary to pipet sample up and down, as the instrument mixes completely. Pipetting up and down can cause loss of beads and reduce yield.

IMPORTANT! Use the volumes described in the following tables. Do not use the volumes that are displayed by the instrument as they are different to ensure effective mixing of viscous samples.

Table 4 Plate setup (KingFisher™ Flex Magnetic Particle Processor 24DW) for 2 mL of plasma

Plate type	Plate position	Plate ID	Reagent	Volume per well
24DW	1	Binding Plate	Digested Plasma	3 mL
			Binding Slurry	1.6 mL
	2	Wash 1 Plate	Wash Solution 1	1 mL
	3	Wash 1 Plate	Wash Solution 1	1 mL
	4	80% Ethanol Plate	80% Ethanol	2 mL
	5	80% Ethanol Plate	80% Ethanol	2 mL
	6	Elution Plate	Elution Solution	30 µL
	7	Tip Comb	24DW Tip Comb	—

Table 5 Plate setup (KingFisher™ Flex Magnetic Particle Processor 24DW) for 4 mL of plasma

Plate type	Plate position	Plate ID	Reagent	Volume per well
24DW	1	Binding Plate 1	Digested Plasma	3 mL
			Binding Slurry	1.6 mL
	2	Binding Plate 2	Digested Plasma	3 mL
			Binding Slurry	1.6 mL
	3	Wash 1 Plate	Wash Solution 1	1 mL
	4	Wash 1 Plate	Wash Solution 1	1 mL
	5	80% Ethanol Plate	80% Ethanol	2 mL
	6	80% Ethanol Plate	80% Ethanol	2 mL
	7	Elution Plate	Elution Solution	30 µL
	8	Tip Comb	24DW Tip Comb	—

Bind, wash, and elute the cfNA

1. Ensure that the instrument is set up for processing with the 24 deep-well magnetic head, then select the program on the instrument according to the following table.

Plasma input volume	Program	Run time
2 mL	**MagMAX_cfNA_Flex_2mL_ALTv1**	32 minutes
4 mL	**MagMAX_cfNA_Flex_4mL_ALTv1**	69 minutes

2. Press **Start** to start the program, then follow the onscreen instructions to load all plates.
3. Press **Start** to start the total nucleic acid extraction.
4. At the end of the run (the instrument beeps), take out the Elution Plate (at the loading position) and place on ice.
5. Press **Start**, then remove all remaining plates when prompted by the instrument.

Store the purified cfNA on ice for immediate use. Alternatively, store the purified cfNA at −20°C or −80°C for long-term storage.

Extract cfNA using KingFisher™ Duo Prime

Prepare cell-free plasma

1. Centrifuge the blood sample at 2000 × *g* for 10 minutes at 4°C.
2. Transfer the plasma to a new centrifuge tube, taking care not to disturb the buffy coat layer.
3. Centrifuge the plasma sample at 16,000 × *g* for 10 minutes at 4°C.
4. Transfer the supernatant to a fresh tube for Proteinase K digestion, noting the volume of plasma.

Digest with Proteinase K

1. Set up Proteinase K digestion in a 50-mL conical tube. Combine reagents in the order that is listed according to the following table.

Proteinase K Digest Component	Cell-free plasma volume	
	2 mL	**4 mL**
MagMAX™ Cell-Free Total Nucleic Acid Proteinase K (20 mg/mL)	30 µL	60 µL
Cell-free plasma	2 mL	4 mL
MagMAX™ Cell-Free Total Nucleic Acid Lysis/Binding Solution	1 mL	2 mL
Total volume	**3.03 mL**	**6.06 mL**

2. Incubate for 30 minutes at 65°C with shaking at 1000 rpm.

3. At the end of Proteinase K incubation, cool the sample on ice for 5 minutes, or until the mix reaches room temperature.

Set up the 24DW processing plates

During Proteinase K incubation, set up the 24DW processing plates outside the instrument according to the following tables.

Note: Ensure that the Binding Slurry is prepared according to Table 3 and well vortexed before adding to the plate. Pipet slowly as solution is viscous. It is not necessary to pipet sample up and down, as the instrument mixes completely. Pipetting up and down can cause loss of beads and reduce yield.

IMPORTANT! Use the volumes described in the following tables. Do not use the volumes that are displayed by the instrument as they are different to ensure effective mixing of viscous samples.

Table 6 Plate setup (KingFisher™ Duo Prime Magnetic Particle Processor 24DW) for 2 mL of plasma

Plate type	Row position	Row ID	Reagent	Volume per well
Plate 1				
24DW	A	Binding	Digested Plasma	3 mL
			Binding Slurry	1.6 mL
	B	Empty	Empty	—
	C	Wash 1	Wash Solution 1	1 mL
	D	Wash 1	Wash Solution 1	1 mL
Plate 2				
24DW	A	80% Ethanol	80% Ethanol	2 mL
	B	80% Ethanol	80% Ethanol	2 mL
	C	Tip Comb	24DW 6-Tip Comb	—
	D	Elution	Elution Solution	30 µL

Table 7 Plate setup (KingFisher™ Duo Prime Magnetic Particle Processor 24DW) for 4 mL of plasma

Plate type	Row position	Row ID	Reagent	Volume per well
Plate 1				
24DW	A	Binding 1	Digested Plasma	3 mL
			Binding Slurry	1.6 mL
	B	Binding 2	Digested Plasma	3 mL
			Binding Slurry	1.6 mL
	C	Wash 1	Wash Solution 1	1 mL
	D	Wash 1	Wash Solution 1	1 mL

Plate type	Row position	Row ID	Reagent	Volume per well
Plate 2				
24DW	A	80% Ethanol	80% Ethanol	2 mL
	B	80% Ethanol	80% Ethanol	2 mL
	C	Tip Comb	24DW 6-Tip Comb	—
	D	Elution	Elution Solution	30 µL

Bind, wash, and elute the cfNA

1. Ensure that the instrument is set up for processing with the 6-pin magnetic head, then select the program on the instrument according to the following table.

plasma input volume	Program	Run time
2 mL	**MagMAX_cfNA_Duo_2mL_ALTv1**	32 minutes
4 mL	**MagMAX_cfNA_Duo_4mL_ALTv1**	70 minutes

2. Press **Start** to start the program, then follow the onscreen instructions to load Plates 1 and 2.
3. Press **Start** to start the total nucleic acid extraction.
4. At the end of the run (the instrument beeps), take out the plates, when instructed, and place on ice.

Store the purified cfNA on ice for immediate use. Alternatively, store the purified cfNA at −20°C or −80°C for long-term storage.

Limited product warranty

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