MagMAX™ Cell-Free Total Nucleic Acid Isolation Kit

Automated or manual isolation of cfNA from 2- or 4-ml plasma samples

Catalog Numbers A36716

Pub. No. MAN0017339 **Rev.** A.0

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 document is intended as a benchtop reference for experienced users of the MagMAX™ Cell-Free Total Nucleic Acid Isolation Kit (Cat. No. A36716). Refer to the *MagMAX™ Cell-Free Total Nucleic Acid Isolation Kit User Guide* (Pub. No. MAN0017274) for detailed instructions.

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 and Rebinding Slurries *(automated protocols only)*

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

- Prepare **Binding Slurry** and **Rebinding Slurry** according to the following tables.

Table 1 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.

Table 2 Rebinding Slurry

Rebinding Slurry Component	Volume per sample	Volume for 10 samples [1]
MagMAX™ Cell-Free Total Nucleic Acid Lysis/Binding Solution	440 µL	5.28 mL
MagMAX™ Cell-Free Total Nucleic Acid Magnetic Beads	10 µL	120 µL
Total volume	**450 µL**	**5.40 mL**

[1] Prepare sufficient Binding Slurry for all samples plus 20% overage.

Note: The volume of Rebinding Slurry is equivalent for all starting plasma volumes and should not be scaled.

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

Process the blood sample

1 Prepare cell-free plasma

a. Centrifuge the blood sample at 2000 × *g* for 10 minutes at 4°C.

b. Transfer the plasma to a new centrifuge tube, taking care not to disturb the buffy coat layer.

c. Centrifuge the plasma sample at 16,000 × *g* for 10 minutes at 4°C.

d. Transfer the supernatant to a fresh tube for Proteinase K digestion, noting the volume of plasma.

2 Digest with Proteinase K

a. 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**

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

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

Extract cfNA using KingFisher™ Flex

1 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 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! Ensure to use the volumes that are 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 3 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	1 mL 80% Ethanol Plate	80% Ethanol	1 mL
	4	500 µL 80% Ethanol Plate	80% Ethanol	500 µL
	5	Elution Plate	Elution Solution	350 µL
	6	Tip Comb	24DW Tip Comb	—

Table 4 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	1 mL 80% Ethanol Plate	80% Ethanol	1 mL
	5	500 µL 80% Ethanol Plate	80% Ethanol	500 µL
	6	Elution Plate	Elution Solution	350 µL
	7	Tip Comb	24DW Tip Comb	—

2 Bind, wash, and elute the cfNA

a. 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_v1**	26 minutes
4 mL	**MagMAX_cfNA_Flex_4mL_v1**	40 minutes

b. Press **Start** to start the program, then follow the onscreen instructions to load all plates.

c. Press **Start** to start the total nucleic acid extraction.

2 Bind, wash, and elute the cfNA *(continued)*

d. At the end of the run (the instrument beeps), take out the Elution Plate (at the loading position) and place on ice.

e. Press **Start**, then remove all remaining plates when prompted by the instrument.

3 Set up the 96DW processing plates

Set up the processing plates outside the instrument according to the following table.

Note: Ensure that the Rebinding Slurry is 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! Ensure to use the volumes that are 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.

Plate type	Plate position	Plate ID	Reagent	Volume per well
96DW	1	Rebinding Plate	First Eluate (from 24DW Plate 6)	350 µL
			Rebind Slurry[1]	450 µL
	2	Wash 1 Plate	Wash Solution 1	1 mL
	3	500 µL 80% Ethanol Plate	80% Ethanol	500 µL
	4	100 µL 80% Ethanol Plate	80% Ethanol	100 µL
Standard KF [2]	5	Elution Plate	Elution Solution	20 µL
96DW	6	Tip Comb	96DW Tip Comb	—

[1] The volume of Rebinding Slurry is equivalent for all starting plasma volumes and should not be scaled.
[2] Kingfisher 96-well microplate.

4 Concentrate the cfNA

a. Ensure that the instrument is set up for processing with the 96 deep-well magnetic head, then select the program **MagMAX_cfNA_Flex_Rebind_v1** on the instrument.

b. Press **Start** to start the program, then follow the onscreen instructions to load all six plates.

c. Press **Start** to start the total nucleic acid concentration.

Note: The **MagMAX_cfNA_Flex_Rebind_v1** program runs for 30 minutes.

d. At the end of the run (the instrument beeps), take out the Elution Plate (at the loading position) and place on ice.

e. 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

1 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 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! Ensure to use the volumes that are 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 5 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	Tip Comb	24DW 6-Tip Comb	
Plate 2				
24DW	A	1 mL 80% Ethanol	80% Ethanol	1 mL
	B	500 µL 80% Ethanol	80% Ethanol	500 µL
	C	Empty	Empty	—
	D	Elution	Elution Solution	350 µL

Table 6 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	Tip Comb	24DW 6-Tip Comb	
Plate 2				
24DW	A	1 mL 80% Ethanol	80% Ethanol	1 mL
	B	500 µL 80% Ethanol	80% Ethanol	500 µL
	C	Empty	Empty	—
	D	Elution	Elution Solution	350 µL

2 Bind, wash, and elute the cfNA

a. 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_v1**	23 minutes
4 mL	**MagMAX_cfNA_Duo_4mL_v1**	40 minutes

b. Press **Start** to start the program, then follow the onscreen instructions to load Plates 1 and 2.

c. Press **Start** to start the total nucleic acid extraction.

d. At the end of the run (the instrument beeps), take out the plates, when instructed, and place on ice.

3 Set up the 96DW processing plate

Set up the processing plate outside the instrument according to the following table.

Note: Ensure that the Rebinding Slurry is 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! Ensure to use the volumes that are described in the following tables. Do not use the volumes that are displayed on the instrument as they are different to ensure effective mixing of viscous samples.

Plate type	Row position	Row ID	Reagent	Volume per well
96DW	A	Rebinding	First eluate (from 24DW Plate 2, Row D)	350 µL
			Rebinding Slurry[1]	450 µL
	B	Wash 1	Wash Solution 1	1 mL
	C	500 µL 80% Ethanol	80% Ethanol	500 µL
	D	100 µL 80% Ethanol	80% Ethanol	100 µL
	E	Tip Comb	96DW 12-Tip Comb	—
	F	Empty	—	—
	G	Empty	—	—
	H	Elution	Elution Solution	20 µL

[1] The volume of Rebinding Slurry is equivalent for all starting plasma volumes and should not be scaled.

4 Concentrate the cfNA

a. Ensure that the instrument is set up for processing with the 12-pin magnetic head, then select the program **MagMAX_cfNA_Duo_Rebind_v1** on the instrument.

b. Press **Start** to start the program, then follow the onscreen instructions to load the plate.

c. Press **Start** to start the total nucleic acid concentration.

Note: The **MagMAX_cfNA_Duo_Rebind_v1** runs for 30 minutes.

d. At the end of the run (the instrument beeps), take out the plate, when instructed, and place on ice.

e. Transfer the eluates into non-stick tubes 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.

Extract cfNA manually

1 Bind cfNA to beads

a. Add MagMAX™ Cell-Free Total Nucleic Acid Lysis/Binding Solution to the digested plasma, according to the following table.

Starting cell-free plasma volume	Lysis/Binding Solution
2 mL	1.5 mL
4 mL	3.0 mL

b. Add well-vortexed MagMAX™ Cell-Free Total Nucleic Acid Magnetic Beads, according to the following table, then vortex briefly to mix.

Starting cell-free plasma volume	Magnetic Beads
2 mL	60 µL
4 mL	120 µL

Note: Ensure that the mixture is homogeneous and the beads are distributed evenly throughout the sample.

c. Place on a shaker for 10 minutes at 1000 rpm to mix and bind the nucleic acid to the beads.

d. Place on the DynaMag™–50 Magnet stand for 5 minutes.

IMPORTANT! Wait the entire 5 minutes before removing supernatant to ensure maximum yield.

e. Remove, then discard the supernatant without disturbing the beads.

2 Wash the beads

a. Resuspend the beads in 1 mL of Wash Solution 1, then mix by pipetting up and down carefully, ensuring all beads are released from the tube walls.

b. Transfer the bead slurry to a new non-stick 1.5-mL microcentrifuge tube and place on the DynaMag™–2 Magnet stand for 20 seconds. Do not discard the 50-mL conical.

c. Collect the supernatant from the bead pellet in the 1.5-mL tube and use it to rinse remaining beads from the 50-mL conical.

d. Carefully transfer the supernatant back to the 1.5-mL tube on the DynaMag™–2 Magnet stand.

e. Leave the 1.5-mL tube on the magnet stand for 2 minutes or until solution clears, then discard the supernatant, being careful not to disturb the beads.

f. Add 1 mL of 80% ethanol.

g. Vortex briefly, then centrifuge briefly.

h. Place on DynaMag™–2 Magnet stand for 2 minutes, or until solution clears.

i. Discard the supernatant.

j. Repeat steps f–i for a second wash with 1 mL of 80% ethanol.

k. Carefully remove, then discard the ethanol.

3 Elute the cfNA

a. Air-dry the beads for 5 minutes, then remove any remaining ethanol with a 10-µL pipette tip.

b. Resuspend the beads in 400 µL of MagMAX™ Cell-Free Total Nucleic Acid Elution Solution and vortex for 5 minutes at high speed.

c. Centrifuge briefly, then place the tube on the DynaMag™–2 Magnet stand to capture beads for 2 minutes.

d. Transfer the supernatant to a new 1.5-mL microcentrifuge tube.

4 Concentrate and wash the cfNA

a. Add 500 µL of MagMAX™ Cell-Free Total Nucleic Acid Lysis/Binding Solution.

b. Add 10 µL of well-vortexed MagMAX™ Cell-Free Total Nucleic Acid Magnetic Beads.

IMPORTANT! Do not use less than 10 µL of beads for the rebinding step.

c. Vortex 1.5-mL tube at high speed for 5 minutes to bind nucleic acid to beads.

d. Briefly centrifuge, then place on DynaMag™–2 Magnet stand for 5 minutes to capture the beads.

e. Discard the supernatant.

f. Add 1 mL of Wash Solution 1.

g. Vortex briefly, then centrifuge briefly.

h. Place on DynaMag™–2 Magnet stand for 2 minutes.

i. Discard the supernatant.

j. Add 1 mL of 80% ethanol.

k. Vortex briefly, then centrifuge briefly.

l. Place on DynaMag™–2 Magnet stand for 2 minutes, or until solution clears.

m. Discard the supernatant.

n. Repeat steps j–m for a second wash with 80% ethanol.

Be careful to remove all the ethanol.

5 Elute the concentrated cfNA

a. Air-dry the beads on the DynaMag™–2 Magnet stand for 3 minutes, taking care not to over-dry the beads.

b. Tap the magnet stand gently against a flat surface to collect remaining liquid, then discard the remaining liquid.

c. Add 15 µL of MagMAX™ Cell-Free Total Nucleic Acid Elution Solution and vortex on high for 5 minutes.

d. Briefly centrifuge, then place on DynaMag™–2 Magnet stand for 2 minutes to capture the beads.

e. Transfer the eluate to a fresh 1.5-mL tube.

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

Manufacturer: Life Technologies Corporation | 2130 Woodward Street | Austin, TX 78744

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0017339

Revision	Date	Description
A.0	12 October 2017	New document instructions for MagMAX™ Cell-Free Total Nucleic Acid Isolation Kit