MagMAX™ Cell-Free DNA Isolation Kit

Isolation of cfDNA from small volumes of plasma and serum samples

Catalog Number A29319

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Note: For safety and biohazard guidelines, see the "Safety" appendix in the *MagMAX™ Cell-Free DNA Isolation Kit User Guide* (Pub. no. MAN0014327). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Applied Biosystems™ MagMAX™ Cell-Free DNA Isolation Kit is designed for isolation of circulating DNA from cell-free human plasma, serum, and urine samples. The kit uses Dynabeads™ MyOne™ SILANE technology and extraction chemistry, ensuring reproducible recovery of high-quality cell-free DNA (cfDNA) that is suitable for a broad range of applications, including sequencing, genotyping, and qPCR.

This guide describes isolation of cfDNA from small volumes of plasma and serum. Two optimized methods are included:

- KingFisher™ Flex Magnetic Particle Processor with 96 Deep Well Head (96DW; 96-well deep-well setting).
- Manual sample processing; 8 individual serum samples can be processed in less than an hour.

The MagMAX™ Cell-Free DNA Isolation Kit is optimized for samples collected in Streck Cell-Free DNA BCT, EDTA, and Acid Citrate Dextrose (ACD) tubes.

Kit contents and storage

Table 1 MagMAX™ Cell-Free DNA Isolation Kit (Cat. no. A29319)

Contents	Amount	Storage
MagMAX™ Cell-Free DNA Magnetic Beads	1.5 mL	2–8°C[1]
MagMAX™ Cell-Free DNA Lysis/Binding Solution	125 mL	15–30°C
MagMAX™ Cell-Free DNA Wash Solution	100 mL	
MagMAX™ Cell-Free DNA Elution Solution	5 mL	

[1] Do not freeze the MagMAX™ Cell Free DNA Magnetic Beads.

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 2 Materials required for cfDNA isolation (all methods)

Item	Source
Equipment	
Thermo Scientific™ Compact Digital Microplate Shaker	Fisher Scientific 11-676-337
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Refrigerated centrifuge, 4°C	MLS
High-speed centrifuge	MLS

Item	Source
Consumables	
Nonstick, RNase-free Microfuge Tubes (1.5 mL)	AM12450
Conical tubes (50 mL)	AM12502
Aerosol-resistant pipette tips	MLS
Reagent reservoirs	MLS
Reagents	
Ethanol, 200 proof (absolute)	MLS
SDS, 20% Solution (required for Proteinase K treatment)	MLS
Proteinase K Solution (20 mg/mL) (required for Proteinase K treatment)	AM2548

Table 3 Additional materials required for automated cfDNA isolation

Item	Source
Magnetic particle processor	
KingFisher™ Flex Magnetic Particle Processor 96DW	5400630
Deep-well plates, one of the following:	
96 Deep-Well Plates for KingFisher™ Flex Magnetic Particle Processor	95040460
MagMAX™ Express-96 Deep Well Plates	4388476
Standard plates, one of the following:	
96 Standard Plates for KingFisher™ Flex Magnetic Particle Processor	97002540
MagMAX™ Express-96 Standard Plates	4388475
Combs, one of the following:	
96 Deep-Well Tip Combs for KingFisher™ Flex Magnetic Particle Processor	97002534
MagMAX™ Express-96 Deep Well Tip Combs	4388487
Consumables	
MicroAmp™ Clear Adhesive Film	4306311

Table 4 Additional materials required for manual cfDNA isolation

Item	Source
Equipment	
Fisher Scientific™ Analog Vortex Mixer	Fisher Scientific 02-215-365
Vortex Adapter	Fisher Scientific NC0070788
mySpin™ 6 Mini Centrifuge	75004061
DynaMag™-2 Magnet	12321D
Eppendorf™ Thermomixer™ C (required for Proteinase K treatment)	Fisher Scientific 05-412-503

If needed, download the KingFisher™ Flex program

1. On the MagMAX™ Cell-Free DNA Isolation Kit web page, scroll down to the **Product Literature** section.
2. Click **cfDNA-600ul-Flex96R_V1** to download the program to your computer.
3. Refer to the manufacturer's documentation for instructions for installing the program on the instrument.

Procedural guidelines

- 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 Plate Cover can be used throughout the procedure, unless it becomes contaminated.
- If you use a titer plate shaker other than the Thermo Scientific™ Compact Digital Microplate Shaker, verify that the plate fits securely on your shaker and test speeds using your specific set up and volumes. Ideal speeds should allow for vigorous mixing without splashing.
- Incubate MagMAX™ Cell Free DNA Lysis/Binding Solution and MagMAX™ Cell Free DNA Wash Solution at 37°C for one hour if precipitates are visible. This can happen if storage temperatures are too low.
- Vortex the MagMAX™ Cell Free DNA Magnetic Beads to fully resuspend them before use.
- We recommend that you prepare master mixes of MagMAX™ Cell Free DNA Lysis/Binding Solution and MagMAX™ Cell Free DNA Magnetic Beads for other sample volumes using the per-mL or the per-well volume and adding 5–10% overage.
- Blood samples collected in the formaldehyde-free preservative contained in the Streck Cell-Free DNA BCT tubes remain stable for up to 14 days. Treating plasma samples in Streck Cell-Free DNA BCT tubes with Proteinase K increases the cfDNA yield up to 50%.
- If you are not using a vortex adaptor that holds tubes horizontally to bind cfDNA to beads in the manual workflow, we recommend that you vortex 20 seconds to ensure thorough mixing, then pulse vortex samples on high speed for the recommended time. Make sure the beads remain in solution to ensure maximum yield.

Isolate cfDNA using the KingFisher™ Flex Magnetic Particle Processor 96DW

1 Prepare cell-free plasma samples

a. Centrifuge the blood samples at 1600 × *g* for 10 minutes at 4°C.
b. Transfer the plasma to a new centrifuge tube.
c. Centrifuge the plasma samples at 16,000 × *g* for 10 minutes at 4°C.

Note: Alternatively, the plasma samples can be centrifuged at 6000 × *g* for 30 minutes to remove any residual blood and cell debris.

2 *(Optional)* Treat the samples with Proteinase K

The Proteinase K treatment is required if you collected your samples in Streck Cell-Free DNA BCT tubes. Otherwise, proceed directly to the next section.

a. Add the following components to a tube in the order indicated.

Reagents	Plasma volume
	600 µL
Proteinase K, 20 mg/mL	12 µL
Plasma sample	600 µL
SDS, 20% Solution[1]	30 µL
Total Volume	642 µL

[1] Do not add SDS directly to the Proteinase K solution, to avoid inactivation of the Proteinase K.

b. Mix well and incubate at 60°C for 20 minutes on the Eppendorf™ Thermomixer™.
c. At the end of the 20-minute incubation, cool the tubes containing the plasma sample to room temperature by placing them on ice for 5 minutes.

3 Set up the processing plates

a. During the centrifugation step or the optional Proteinase K treatment, set up the processing plates outside the instrument as described in the following table.

Table 5 Plate setup (KingFisher™ Flex Magnetic Particle Processor 96DW) for 600 µL of plasma

Plate ID	Plate position[1]	Reagent	Volume per well
Sample Plate 1	1	MagMAX™ Cell Free DNA Lysis/Binding Solution	375 µL
		MagMAX™ Cell Free DNA Magnetic Beads	6 µL
Sample Plate 2	2	MagMAX™ Cell Free DNA Lysis/Binding Solution	375 µL
		MagMAX™ Cell Free DNA Magnetic Beads	6 µL
Wash Plate 1	3	MagMAX™ Cell Free DNA Wash Solution	1 mL
Wash Plate 2	4	80% Ethanol	1 mL
Wash Plate 3	5	80% Ethanol	500 µL
Elution Plate	6	MagMAX™ Cell Free DNA Elution Solution	30–50 µL
Tip Comb	7	Place a Deep-Well Comb in a plate.	

[1] Position on the instrument

IMPORTANT! Make sure that you are using a Standard Plate as Elution Plate. Use Deep-Well Plates for all the other positions.

b. Gently shake Sample Plates 1 and 2 to mix the reagents.
c. Add 300 µL of plasma sample to the wells of Sample Plates 1 and 2.

4 Bind, wash, and elute the cfDNA

a. Ensure that the instrument is set up for processing with the 96-well deep-well magnetic head, and select the program **cfDNA-600ul-Flex96R_V1** on the instrument.
b. Start the run and load the prepared processing plates in their positions when prompted by the instrument (see Table 5).
c. At the end of the run (approximately 43 minutes after the initial start), remove the Elution Plate from the instrument and cover it immediately.

IMPORTANT! To prevent evaporation and contamination, do not allow the purified samples to sit uncovered at room temperature for more than 10 minutes.

The purified cfDNA is ready for immediate use. Alternatively, store the covered Elution Plate:

- On ice for up to 24 hours.
- At –20°C for long-term storage.

Isolate cfDNA manually

1 Prepare cell-free plasma samples

a. Centrifuge the blood samples at 1600 × *g* for 10 minutes at 4°C.
b. Transfer the plasma to a new centrifuge tube.
c. Centrifuge the plasma samples at 16,000 × *g* for 10 minutes at 4°C.

Note: Alternatively, the plasma samples can be centrifuged at 6000 × *g* for 30 minutes to remove any residual blood and cell debris.

Proceed to the next step according the collection tubes you are using.

Type of collection tube	Proceed to...
Streck Cell-Free DNA BCT	"Option 1: Lyse the plasma samples (with PK) and bind the cfDNA to the beads" on page 3
Others	"Option 2: Lyse the plasma samples (without PK) and bind the cfDNA to the beads" on page 4

2 Option 1: Lyse the plasma samples (with PK) and bind the cfDNA to the beads

The Proteinase K treatment is required if you collected your samples in Streck Cell-Free DNA BCT tubes. Otherwise, proceed directly to the next section.

a. Add the following components to a tube in the order indicated.

Reagents	Plasma volume				
	100 µL	**200 µL**	**400 µL**	**500 µL**	**600 µL**
Proteinase K, 20 mg/mL	2 µL	3 µL	6 µL	8 µL	12 µL
Plasma sample	100 µL	200 µL	400 µL	500 µL	600 µL
SDS, 20% Solution[1]	5 µL	10 µL	20 µL	25 µL	30 µL
Total Volume	107 µL	213 µL	426 µL	533 µL	642 µL

[1] Do not add SDS directly to the Proteinase K solution, to avoid inactivation of the Proteinase K.

b. Mix well and incubate at 60°C for 20 minutes on the Eppendorf™ Thermomixer™.
c. During the incubation, prepare the Binding Solution/Beads Mix according to the following table and mix well.

Reagents	Plasma volume				
	100 µL	**200 µL**	**400 µL**	**500 µL**	**600 µL**
MagMAX™ Cell Free DNA Lysis/Binding Solution	150 µL	300 µL	500 µL	630 µL	750 µL
MagMAX™ Cell Free DNA Magnetic Beads	5 µL		10 µL		
Total Volume	155 µL	305 µL	510 µL	640 µL	760 µL

d. At the end of the 20-minute incubation, cool the tubes containing the plasma sample to room temperature by placing them on ice for 5 minutes.
e. Add the prepared Binding Solution/Beads Mix to each sample according to the following table.

Reagents	Plasma volume				
	100 µL	**200 µL**	**400 µL**	**500 µL**	**600 µL**
Binding Solution/Beads Mix	155 µL	305 µL	510 µL	640 µL	760 µL

f. Place the tube horizontally on the vortex adapator and shake for 10 minutes at medium speed to bind the cfDNA to the beads.
Alternatively, pulse vortexing may be employed, ensuring that the beads stay in solution (see "Procedural guidelines" on page 2).
g. Centrifuge for a few seconds to collect all liquid at the bottom of the tube.
h. Place the tube on the appropriate DynaMag™ Magnet for 5 minutes or until the solution clears and the beads are pelleted against the magnet.
i. Carefully discard the supernatant with a pipette.
j. Keep the tube on the magnet for another minute and remove the residual supernatant with a pipette.
k. Proceed directly to step 4a of "Wash with Wash Solution" on page 4.

3 **Option 2: Lyse the plasma samples (without PK) and bind the cfDNA to the beads**

a. Prepare the Binding Solution/Beads Mix according to the following table and mix thoroughly.

Reagents	Plasma volume				
	100 µL	**200 µL**	**400 µL**	**500 µL**	**600 µL**
MagMAX™ Cell Free DNA Lysis/Binding Solution	150 µL	300 µL	500 µL	630 µL	750 µL
MagMAX™ Cell Free DNA Magnetic Beads	5 µL		10 µL		
Total Volume	155 µL	305 µL	510 µL	640 µL	760 µL

b. Add the appropriate volume of plasma sample.

c. Place the tube horizontally on the vortex adapator and shake for 10 minutes at medium speed to bind the cfDNA to the beads.

Alternatively, pulse vortexing may be employed, ensuring that the beads stay in solution (see "Procedural guidelines" on page 2).

d. Centrifuge for a few seconds to collect all liquid at the bottom of the tube.

e. Place the tube on the appropriate DynaMag™ Magnet for 5 minutes or until the solution clears and the beads are pelleted against the magnet.

f. Carefully discard the supernatant with a pipette.

g. Keep the tube on the magnet for another minute and remove the residual supernatant with a pipette.

4 **Wash with Wash Solution**

a. Remove the tube from the DynaMag™-2 Magnet, then resuspend the beads in 500 µL of MagMAX™ Cell Free DNA Wash Solution.

b. Vortex for 30 seconds.

c. Centrifuge for a few seconds to collect all liquid at the bottom of the tube.

d. Place the tube on the DynaMag™-2 Magnet for 2 minutes, or until the solution clears and the beads are pelleted against the magnets.

e. Carefully discard the supernatant with a pipette.

f. Keeping the tube on the DynaMag™-2 Magnet, tap the magnet stand on the benchtop 5 times, then remove any residual liquid with a 200-µL pipette.

5 **Wash twice with 80% ethanol**

a. Remove the tube from the DynaMag™-2 Magnet, add 500 µL of 80% ethanol, then vortex for 30 seconds.

b. Centrifuge for a few seconds to collect all liquid at the bottom of the tube.

c. Place the tube on the DynaMag™-2 Magnet for 1 minute, or until the solution clears and the beads are pelleted against the magnets.

d. Remove the supernatant with a 1-mL pipette.

e. Keeping the tube on the DynaMag™-2 Magnet, tap the magnet stand on the benchtop 5 times, then remove any residual liquid with a 200-µL pipette.

f. Repeat step 5a–step 5d for a second wash with 80% ethanol.

g. Keeping the tube on the DynaMag™-2 Magnet, air dry the beads for 2–3 minutes.

h. Keeping the tube on the DynaMag™-2 Magnet, tap the magnet stand on the benchtop 5 times, then remove any residual liquid with a 200-µL pipette.

6 **Elute the cfDNA**

a. Add 10–50 µL of MagMAX™ Cell Free DNA Elution Solution to the tube.

b. Place the tube horizontally on the vortex adapator and shake for 5 minutes at medium speed.

Alternatively, pulse vortexing may be employed, ensuring that the beads stay in solution (see "Procedural guidelines" on page 2).

c. Centrifuge for a few seconds to collect all liquid at the bottom of the tube.

d. Place the tube on the DynaMag™-2 Magnet for 2 minutes, or until the solution clears and the beads are pelleted against the magnets.

The supernatant contains the purified cfDNA.

The purified cfDNA is ready for immediate use. Alternatively, transfer the supernatant to a new microcentrifuge tube and store:

- At 4°C for up to 24 hours.
- At –20°C for long-term storage.

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Revision	Date	Description
B.0	10 May 2016	Minor correction in the title of the automated isolation section.
A.0	25 March 2016	New document

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