MagMAX™ Cell-Free DNA Isolation Kit

USER GUIDE

Isolation of cfDNA from plasma and serum samples

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ThermoFisher SCIENTIFIC

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Revision history: Revision history of Pub. no. MAN0014327

Revision	Date	Description
C.0	04 August 2016	Changes in guidelines for handling 10-mL samples
B.0	25 March 2016	Addition of a shaking step to bind cfDNA to the beads in the manual isolation protocol
A.0	2 September 2015	New document

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Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

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 plasma and serum samples. Three optimized methods are included:

- KingFisher™ Flex Magnetic Particle Processor with 24 Deep Well Head (24DW; 24-well deep-well setting).
- KingFisher™ Duo Prime Magnetic Particle Processor (6-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.

Note:

- **For downstream applications using AmpliSeq™ technology:** refer to the protocols described in Appendix B, "Alternate protocol for isolation of higher concentration cfDNA".
- **For downstream applications using Ion Torrent™ technology:** refer to the protocols described in Appendix B, "Alternate protocol for isolation of higher concentration cfDNA".
- **For downstream applications requiring more concentrated cfDNA in smaller elution volumes**: refer to the protocols described in Appendix B, "Alternate protocol for isolation of higher concentration cfDNA".
- **For smaller volumes of plasma and serum samples:** refer to *MagMAX™ Cell-Free DNA Isolation Kit User Bulletin (small volumes of plasma and serum samples)* (Pub. no. MAN0015629).
- **For urine samples:** refer to *MagMAX™ Cell-Free DNA Isolation Kit User Bulletin (urine samples)* (Pub. no. MAN0015628).

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
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

Item	Source
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, one of the following:	
KingFisher™ Flex Magnetic Particle Processor with 24 Deep Well Head	5400640
KingFisher™ Duo Prime Magnetic Particle Processor	5400110
Plates and combs	
KingFisher™ Flex 24 Deep-Well Plates (compatibles with both magnetic particle processors)	95040480
Tip comb, compatible with the magnetic particle processor used:	
KingFisher™ Flex 24 Deep Well Tip Comb and plate	97002610
6-Tip Combs for KingFisher™ Duo Prime Magnetic Particle Processor	97002610
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
DynaMag™-50 Magnet	12302D
DynaMag™-2 Magnet	12321D
Eppendorf™ Thermomixer™ C (required for Proteinase K treatment)	Fisher Scientific 05-412-503
Reagent	
10X TAE Buffer (required for the alternate protocol for isolation of higher concentration cfDNA)	AM9869

If needed, download the KingFisher™ Flex or Duo program

1. On the MagMAX™ Cell-Free DNA Isolation Kit web page, scroll down to the **Product Literature** section.
2. Click on the appropriate program file for your sample size to download the program to your computer:

Instrument	For a total plasma/serum volume of:	
	2 mL	4 or 5 mL
KingFisher™ Flex Magnetic Particle Processor 24DW	**MagMAX cfDNA-2mL-Flex**	**MagMAX cfDNA-4mL-Flex**
KingFisher™ Duo Prime Magnetic Particle Processor	**MagMAX cfDNA-2mL-DUO**	**MagMAX cfDNA-4mL-DUO**

3. Refer to the manufacturer's documentation for instructions for installing the program on the instrument.

Methods

Note:

- **For downstream applications using AmpliSeq™ technology:** refer to the protocols described in Appendix B, "Alternate protocol for isolation of higher concentration cfDNA".
- **For downstream applications using Ion Torrent™ technology:** refer to the protocols described in Appendix B, "Alternate protocol for isolation of higher concentration cfDNA".

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.
- When performing manual isolations, we recommend that you use capped tubes with enough volume left unfilled to allow for vigorous mixing. We recommend that you secure 10– or 50–mL screw-cap conical tubes to the Thermo Scientific™ Compact Digital Microplate Shaker and shake at speed 7 for the binding step.
- 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%.

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

Prepare cell-free plasma samples

1. Centrifuge the blood samples at 2000 × *g* for 10 minutes at 4°C.
2. Transfer the plasma to a new centrifuge tube.
3. 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.

(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.

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

Reagents	Plasma volume[1]		
	1 mL	2 mL	4 mL
Proteinase K, 20 mg/mL	15 µL	30 µL	60 µL
Plasma sample	1 mL	2 mL	4 mL
SDS, 20% Solution[2]	50 µL	100 µL	200 µL
Total Volume	1.065 mL	2.13 mL	4.26 mL

[1] For volumes larger than 5 mL, contact Technical Support.
[2] Do not add SDS directly to the Proteinase K solution, to avoid inactivation of the Proteinase K.

2. Mix well and incubate at 60°C for 20 minutes on the Eppendorf™ Thermomixer™.
3. 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.

Set up the processing plates

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

Table 5 Plate setup (KingFisher™ Flex Magnetic Particle Processor 24DW) for 2 mL of plasma

Plate ID	Plate position[1]	Reagent	Volume per well
Sample Plate 1	1	MagMAX™ Cell Free DNA Lysis/Binding Solution	2.5 mL
		MagMAX™ Cell Free DNA Magnetic Beads	30 µL
Wash Plate 1	2	MagMAX™ Cell Free DNA Wash Solution	1 mL
Wash Plate 2	3	MagMAX™ Cell Free DNA Wash Solution	1 mL

Plate ID	Plate position[1]	Reagent	Volume per well
Wash Plate 3	4	80% Ethanol	2 mL
Wash Plate 4	5	80% Ethanol	500 µL
Elution Plate	6	MagMAX™ Cell Free DNA Elution Solution	50–100 µL
Tip Comb	7	Place a Deep-Well Comb in a plate.	

[1] Position on the instrument

Table 6 Plate setup (KingFisher™ Flex Magnetic Particle Processor 24DW) for 4 or 5 mL of plasma

Plate ID	Plate position[1]	Reagent	Volume per well	
			4 mL of plasma	5 mL of plasma
Sample Plate 1	1	MagMAX™ Cell Free DNA Lysis/Binding Solution	2.5 mL	3 mL
		MagMAX™ Cell Free DNA Magnetic Beads	30 µL	37 µL
Sample Plate 2	2	MagMAX™ Cell Free DNA Lysis/Binding Solution	2.5 mL	3 mL
		MagMAX™ Cell Free DNA Magnetic Beads	30 µL	37 µL
Wash Plate 1	3	MagMAX™ Cell Free DNA Wash Solution	1 mL	
Wash Plate 2	4	MagMAX™ Cell Free DNA Wash Solution	1 mL	
Wash Plate 3	5	80% Ethanol	2 mL	
Wash Plate 4	6	80% Ethanol	500 µL	
Elution Plate	7	MagMAX™ Cell Free DNA Elution Solution	50–100 µL	
Tip Comb	8	Place a Deep-Well Comb in a plate.		

[1] Position on the instrument

2. Gently shake Sample Plates 1 and 2 (if applicable) to mix the reagents.

3. Add plasma sample to the wells of Sample Plates 1 and 2, if applicable, according to the following table.

For a total plasma/serum volume of:	Add this volume of plasma/serum to:	
	Sample Plate 1	Sample Plate 2
2 mL	2 mL	N/A
4 mL	2 mL	2 mL
5 mL	2.5 mL	2.5 mL

Bind, wash, and elute the cfDNA

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

For a total plasma/serum volume of:	Program
2 mL	**MagMAX cfDNA-2mL-Flex**
4 mL	**MagMAX cfDNA-4mL-Flex**
5 mL	**MagMAX cfDNA-4mL-Flex**

2. Start the run and load the prepared processing plates in their positions when prompted by the instrument (see Table 5 or Table 6).

3. At the end of the run (approximately 35 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 using the KingFisher™ Duo Prime Magnetic Particle Processor

Prepare cell-free plasma samples

1. Centrifuge the blood samples at 2000 × *g* for 10 minutes at 4°C.

2. Transfer the plasma to a new centrifuge tube.

3. 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.

(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.

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

Reagents	Plasma volume[1]		
	1 mL	2 mL	4 mL
Proteinase K, 20 mg/mL	15 µL	30 µL	60 µL
Plasma sample	1 mL	2 mL	4 mL
SDS, 20% Solution[2]	50 µL	100 µL	200 µL
Total Volume	1.065 mL	2.13 mL	4.26 mL

[1] For volumes larger than 5 mL, contact Technical Support.
[2] Do not add SDS directly to the Proteinase K solution, to avoid inactivation of the Proteinase K.

2. Mix well and incubate at 60°C for 20 minutes on the Eppendorf™ Thermomixer™.

3. 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.

Set up the processing plates

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

Table 7 Plate setup (KingFisher™ Duo Prime instrument)

Plate	Row ID	Plate row[1]	Reagent	Volume per well		
				2 mL of plasma	4 mL of plasma	5 mL of plasma
1	Sample 1	A	MagMAX™ Cell Free DNA Lysis/Binding Solution	2.5 mL	2.5 mL	3 mL
			MagMAX™ Cell Free DNA Magnetic Beads	30 µL	30 µL	37 µL
	Sample 2	B	MagMAX™ Cell Free DNA Lysis/Binding Solution	Leave empty[2]	2.5 mL	3 mL
			MagMAX™ Cell Free DNA Magnetic Beads		30 µL	37 µL
	Wash 1	C	MagMAX™ Cell Free DNA Wash Solution	1 mL		
	Wash 2	D	MagMAX™ Cell Free DNA Wash Solution	1 mL		
2	Elution	A	MagMAX™ Cell Free DNA Elution Solution	50–100 µL		
	Low Vol. Wash	B	80% Ethanol	500 µL		
	High Vol. Wash	C	80% Ethanol	2 mL		
	Tip Comb	D	Place a Deep-Well Tip Comb in Row D.			

[1] Row on the Deep-Well Plate
[2] See following table

2. Gently shake Plate 1 to mix the reagents.

3. Add plasma sample to the wells of Row A and B, if applicable, of Plate 1 according to the following table.

For a total plasma/serum volume of:	Add this volume of plasma/serum to:	
	Row A	Row B
2 mL	2 mL	N/A
4 mL	2 mL	2 mL
5 mL	2.5 mL	2.5 mL

Bind, wash, and elute the cfDNA

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

For a total plasma/serum volume of:	Program
2 mL	**MagMAX cfDNA-2mL-DUO**
4 mL	**MagMAX cfDNA-4mL-DUO**
5 mL	**MagMAX cfDNA-4mL-DUO**

2. Start the run and load the prepared processing plates when prompted by the instrument (see Table 7).

   Plate 2 is loaded before Plate 1.

3. At the end of the run (approximately 35 minutes after initial start), remove the two plates from the instrument and transfer the eluted cfDNA (Row A of Plate 2) to an Elution Plate.

4. Cover the plate 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

Workflow

Prepare cell-free plasma samples

▼

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

Follow this procedure for plasma samples collected in Streck Cell-free DNA BCT tubes.

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

▼

Wash with Wash Solution

▼

Wash twice with 80% ethanol

▼

Elute the cfDNA

Prepare cell-free plasma samples

1. Centrifuge the blood samples at 2000 × *g* for 10 minutes at 4°C.
2. Transfer the plasma to a new centrifuge tube.
3. 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
Others	Option 2: Lyse the plasma samples (without PK) and bind the cfDNA to the beads

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.

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

Reagents	Plasma volume			
	1 mL	**2 mL**	**4 mL**	**10 mL**
Proteinase K, 20 mg/mL	15 µL	30 µL	60 µL	150 µL
Plasma sample	1 mL	2 mL	4 mL	10 mL
SDS, 20% Solution[1]	50 µL	100 µL	200 µL	500 µL
Total Volume	1.065 mL	2.13 mL	4.26 mL	10.65 mL

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

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

Reagents	Plasma volume			
	1 mL	**2 mL**	**4 mL**	**10 mL**
MagMAX™ Cell Free DNA Lysis/Binding Solution	1.25 mL	2.5 mL	5 mL	12.5 mL
MagMAX™ Cell Free DNA Magnetic Beads	15 µL	30 µL	60 µL	150 µL
Total Volume	1.265 mL	2.53 mL	5.06 mL	12.65 mL

4. 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.

5. Add the prepared Binding Solution/Beads Mix to each sample according to the following table.

Reagents	Plasma volume			
	1 mL	2 mL	4 mL	10 mL
Binding Solution/Beads Mix	1.265 mL	2.53 mL	5.06 mL	12.65 mL

6. Thoroughly mix the plasma sample and the Binding Solution/Beads Mix by swirling or by inverting the tube 10 times.

7. Shake vigorously for 10 minutes on a vortex with tube adaptor or the microtiter plate shaker (speed 7 or higher) to bind the cfDNA to the beads.

   IMPORTANT! Make sure to vigorously shake to ensure optimal cfDNA binding to the beads. Insufficient shaking will result in lower cfDNA recovery yield.

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

9. Carefully discard the supernatant with a pipette.

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

11. Proceed directly to step 1 of "Wash with Wash Solution" on page 17.

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

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

Reagents	Plasma volume			
	1 mL	2 mL	4 mL	10 mL
MagMAX™ Cell Free DNA Lysis/Binding Solution	1.25 mL	2.5 mL	5 mL	12.5 mL
MagMAX™ Cell Free DNA Magnetic Beads	15 µL	30 µL	60 µL	150 µL
Total Volume	1.265 mL	2.53 mL	5.06 mL	12.65 mL

2. Add the appropriate volume of plasma sample.

3. Thoroughly mix the plasma sample and the Binding Solution/Beads Mix by swirling or by inverting the tube 10 times.

4. Shake vigorously for 10 minutes on a vortex with tube adaptor or the microtiter plate shaker (speed 7 or higher) to bind the cfDNA to the beads.

   IMPORTANT! Make sure to vigorously shake to ensure optimal cfDNA binding to the beads. Insufficient shaking will result in lower cfDNA recovery yield.

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

6. Carefully discard the supernatant with a pipette.
7. Keep the tube on the magnet for another minute and remove the residual supernatant with a pipette.

Wash with Wash Solution

1. Resuspend the beads in 1 mL of MagMAX™ Cell Free DNA Wash Solution.
2. Transfer the bead slurry to a new non-stick 1.5-mL microcentrifuge tube and save the lysis/binding tube.
3. Place the microcentrifuge tube containing the bead slurry on the DynaMag™-2 Magnet for 20 seconds.
4. Collect and use the supernatant of the bead slurry to rinse the saved lysis/binding microcentrifuge tube.
5. Transfer any residual beads to the tube containing the bead slurry and discard the lysis/binding tube.
6. Leave the tube on the DynaMag™-2 Magnet for an additional 2 minutes, or until the solution clears and the beads are pelleted against the magnets.
7. Remove the supernatant with a 1-mL pipette.
8. 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.
9. Remove the tube from the DynaMag™-2 Magnet, add 1 mL of MagMAX™ Cell Free DNA Wash Solution, then vortex for 30 seconds.
10. Place the tube on the DynaMag™-2 Magnet for 2 minutes, or until the solution clears and the beads are pelleted against the magnets.
11. Remove the supernatant with a 1-mL pipette.
12. 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.

Wash twice with 80% ethanol

1. Remove the tube from the DynaMag™-2 Magnet, add 1 mL of 80% ethanol, then vortex for 30 seconds.
2. Place the tube on the DynaMag™-2 Magnet for 2 minutes, or until the solution clears and the beads are pelleted against the magnets.
3. Remove the supernatant with a 1-mL pipette.
4. 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. Repeat step 1–step 3 for a second wash with 80% ethanol.

6. Keeping the tube on the DynaMag™-2 Magnet, air dry the beads for 3–5 minutes.

7. 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.

Elute the cfDNA

1. Add MagMAX™ Cell Free DNA Elution Solution to the tube according to the following table.

Reagents	Plasma volume			
	1 mL	2 mL	4 mL	10 mL
MagMAX™ Cell Free DNA Elution Solution	15 µL	30 µL	50–60 µL	100–150 µL

2. Vortex for 5 minutes using a vortex adapter.

3. 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.

A

Troubleshooting

Observation	Possible cause	Recommended action
Lower yield than expected	The MagMAX™ Cell Free DNA Magnetic Beads were not properly stored	Remove the MagMAX™ Cell Free DNA Magnetic Beads from the kit and store them at 2–8°C. Do not freeze the beads.
		Allow the beads to warm to room temperature before use.
	An insufficient amount of MagMAX™ Cell Free DNA Magnetic Beads was added	Vortex the tube containing the magnetic beads thoroughly immediately before use.
		If you are preparing a master mix of magnetic beads and MagMAX™ Cell Free DNA Lysis/Binding Solution, ensure that the mix is homogeneous before adding sample to the mixture.
	The MagMAX™ Cell Free DNA Magnetic Beads are not optimally dried	Drying times may vary depending on the amount of beads used and the environment. Lower volumes of beads require less time to dry. Airflow and humidity in the immediate environment may shorten or lengthen the optimal bead drying time.
		Overdried beads will stick to the wall of the plastics and be difficult to re-suspend.
		Underdried beads may carry ethanol into the eluate and negatively impact downstream applications.
	The sample contains low levels of cfDNA	Increase the starting sample volume.

Observation	Possible cause	Recommended action
Lower yield than expected *(continued)*	The desired target is >1kb	After the magnetic beads are air-dried in step 6 of "Wash twice with 80% ethanol" on page 17: 1. Add 25 µL 2mM NaOH to the bound dried magnetic beads. 2. Close cap and tap to fully disperse the beads. 3. Heat at 56°C for 3 minutes, then vortex for 2 minutes. Alternatively, use preheated NaOH in step 1 above and vortex for 3 minutes. 4. Quickly spin in a mini-centrifuge to bring down the liquid drops off the tube cap. 5. Add 25 µL of MagMAX™ Cell Free DNA Elution Solution and vortex 3 minutes. 6. Place the tube back on the magnetic stand 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.
	Insufficient mixing of the samples with the magnetic beads during the binding step of the manual cfDNA isolation	After adding your sample to the tube containing the MagMAX™ Cell Free DNA Lysis/Binding Solution and the magnetic beads, screw tightly the cap of the conical tube, secure the tubes to the Thermo Scientific™ Compact Digital Microplate Shaker, then shake at speed 7 for the binding step. Alternatively, mix vigorously using a vortex set on maximum speed for 10 minutes using a vortex tube adapter.
Magnetic bead carryover	Loose beads present in the eluate or inadvertently transferred	Be sure to leave the tube on the magnetic stand when removing the eluate containing the cfDNA.
		If beads are carried over into the new tube, place the tube on the magnetic stand again, wait for the beads to pellet and then transfer the sample to another tube.
Abundance of gDNA in eluate	Hemolytic plasma, lipemic plasma, or other compromised sample types (see Figure 1)	Yields from these types of samples vary greatly from donor to donor. We recommend processing these types of samples using the manual protocol.

Observation	Possible cause	Recommended action
Variations in cfDNA yield from donor to donor	Variation in amount of circulating cfDNA. Levels of cfDNA in circulation can range from 1 to 100 ng/mL of plasma or serum depending on the donor.	For samples containing low levels of cfDNA, increase the starting sample volume.

Example of Agilent™ High Sensitivity Analysis traces

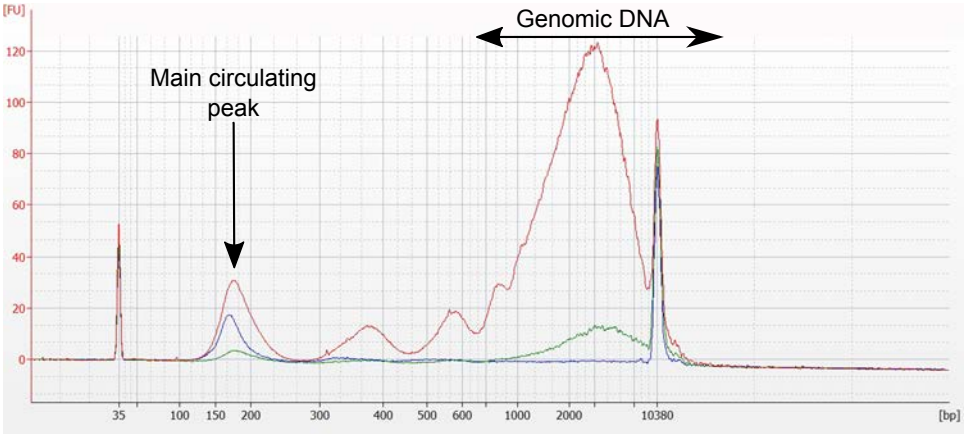

Figure 1 Agilent™ High Sensitivity Analysis on 1 µL of cfDNA isolated from 2 mL of plasma with the MagMAX™ Cell-Free DNA Isolation Kit

The blue trace is from a normal plasma sample, the green trace is from a lipemic plasma sample, and the red trace is from a severely hemolysed plasma sample.

B Alternate protocol for isolation of higher concentration cfDNA

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.
- 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 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%.

Isolate cfDNA manually

Prepare cell-free plasma samples

1. Centrifuge the blood samples at 2000 × *g* for 10 minutes at 4°C.
2. Transfer the plasma to a new centrifuge tube.
3. 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 23
Others	"Option 2: Lyse the plasma samples (without PK) and bind the cfDNA to the beads" on page 24

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.

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

Reagents	Plasma volume			
	1 mL	2 mL	4 mL	10 mL
Proteinase K, 20 mg/mL	15 µL	30 µL	60 µL	150 µL
Plasma sample	1 mL	2 mL	4 mL	10 mL
SDS, 20% Solution[1]	50 µL	100 µL	200 µL	500 µL
Total Volume	1.065 mL	2.13 mL	4.26 mL	10.65 mL

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

2. Mix well and incubate at 60°C for 20 minutes on the Eppendorf™ Thermomixer™.

3. During the incubation, prepare the Binding Solution/Beads Mix according to the following table and mix well.

Reagents	Plasma volume			
	1 mL	2 mL	4 mL	10 mL
MagMAX™ Cell Free DNA Lysis/Binding Solution	1.25 mL	2.5 mL	5 mL	12.5 mL
MagMAX™ Cell Free DNA Magnetic Beads	15 µL	30 µL	60 µL	150 µL
Total Volume	1.265 mL	2.53 mL	5.06 mL	12.65 mL

4. 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.

5. Add the prepared Binding Solution/Beads Mix to each sample according to the following table.

Reagents	Plasma volume			
	1 mL	2 mL	4 mL	10 mL
Binding Solution/Beads Mix	1.265 mL	2.53 mL	5.06 mL	12.65 mL

6. Thoroughly mix the plasma sample and the Binding Solution/Beads Mix by swirling or by inverting the tube 10 times.

7. Shake vigorously for 10 minutes on a vortex with tube adaptor or the microtiter plate shaker (speed 7 or higher) to bind the cfDNA to the beads.

   IMPORTANT! Make sure to vigorously shake to ensure optimal cfDNA binding to the beads. Insufficient shaking will result in lower cfDNA recovery yield.

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

9. Carefully discard the supernatant with a pipette.

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

11. Proceed directly to step 1 of "Wash with Wash Solution and 80% ethanol" on page 25.

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

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

Reagents	Plasma volume			
	1 mL	2 mL	4 mL	10 mL
MagMAX™ Cell Free DNA Lysis/Binding Solution	1.25 mL	2.5 mL	5 mL	12.5 mL
MagMAX™ Cell Free DNA Magnetic Beads	15 µL	30 µL	60 µL	150 µL
Total Volume	1.265 mL	2.53 mL	5.06 mL	12.65 mL

2. Add the appropriate volume of plasma sample.

3. Thoroughly mix the plasma sample and the Binding Solution/Beads Mix by swirling or by inverting the tube 10 times.

4. Shake vigorously for 10 minutes on a vortex with tube adaptor or the microtiter plate shaker (speed 7 or higher) to bind the cfDNA to the beads.

   IMPORTANT! Make sure to vigorously shake to ensure optimal cfDNA binding to the beads. Insufficient shaking will result in lower cfDNA recovery yield.

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

6. Carefully discard the supernatant with a pipette.

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

Wash with Wash Solution and 80% ethanol

1. Resuspend the beads in 1 mL of MagMAX™ Cell Free DNA Wash Solution.
2. Transfer the bead slurry to a new non-stick 1.5-mL microcentrifuge tube and save the lysis/binding tube.
3. Place the microcentrifuge tube containing the bead slurry on the DynaMag™-2 Magnet for 20 seconds.
4. Collect and use the supernatant of the bead slurry to rinse the saved lysis/binding microcentrifuge tube.
5. Transfer any residual beads to the tube containing the bead slurry and discard the lysis/binding tube.
6. Leave the tube on the DynaMag™-2 Magnet for an additional 2 minutes, or until the solution clears and the beads are pelleted against the magnets.
7. Remove the supernatant with a 1-mL pipette.
8. 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.
9. Remove the tube from the DynaMag™-2 Magnet, add 1 mL of 80% ethanol, then vortex for 30 seconds.
10. Place the tube on the DynaMag™-2 Magnet for 2 minutes, or until the solution clears and the beads are pelleted against the magnets.
11. Remove the supernatant with a 1-mL pipette.
12. Keeping the tube on the DynaMag™-2 Magnet, air dry the beads for 3–5 minutes.
13. 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.

Elute the cfDNA, rebind, and wash

1. Add 400 µL of 0.1X TAE and vortex for 5 minutes.
2. Leave the tube on the DynaMag™-2 Magnet for an additional 2 minutes, or until the solution clears and the beads are pelleted against the magnets.
3. Transfer the supernatant to a new 1.5-mL microcentrifuge tube.
4. Add 5–10 µL of MagMAX™ Cell Free DNA Magnetic Beads and 500 µL of MagMAX™ Cell Free DNA Lysis/Binding Solution to the supernatant and mix thoroughly.

   Note: We recommend that you use 10 µL of MagMAX™ Cell Free DNA Magnetic Beads for 10-mL samples.
5. Shake for 5 minutes to bind cfDNA to beads.
6. Place the tube on the DynaMag™-2 Magnet for 5 minutes, or until the solution clears and the beads are pelleted against the magnets.

7. Remove the supernatant with a 1-mL pipette.
8. Remove the tube from the DynaMag™-2 Magnet, add 1 mL of MagMAX™ Cell Free DNA Wash Solution, then vortex for 30 seconds.
9. Place the tube on the DynaMag™-2 Magnet for 2 minutes, or until the solution clears and the beads are pelleted against the magnets.
10. Remove the supernatant with a 1-mL pipette.
11. 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.

Wash twice with 80% ethanol

1. Remove the tube from the DynaMag™-2 Magnet, add 1 mL of 80% ethanol, then vortex for 30 seconds.
2. Place the tube on the DynaMag™-2 Magnet for 2 minutes, or until the solution clears and the beads are pelleted against the magnets.
3. Remove the supernatant with a 1-mL pipette.
4. 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. Repeat step 1–step 3 for a second wash with 80% ethanol.
6. Keeping the tube on the DynaMag™-2 Magnet, air dry the beads for 3–5 minutes.
7. 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.

Elute the cfDNA

1. Add 15 µL of MagMAX™ Cell Free DNA Elution Solution to the tube.
2. Vortex for 5 minutes using a vortex adapter.
3. 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.

C

Yield and quality measurement

cfDNA quantification

We recommend using the Agilent™ High Sensitivity DNA Analysis Kit (Cat. no. 5067–4626) to quantify the cfDNA fraction. cfDNA is fragmented dsDNA with a major peak around 170 bp (see Figure 2). The sensitivity of the kit is 100 pg/µL for fragmented DNA.

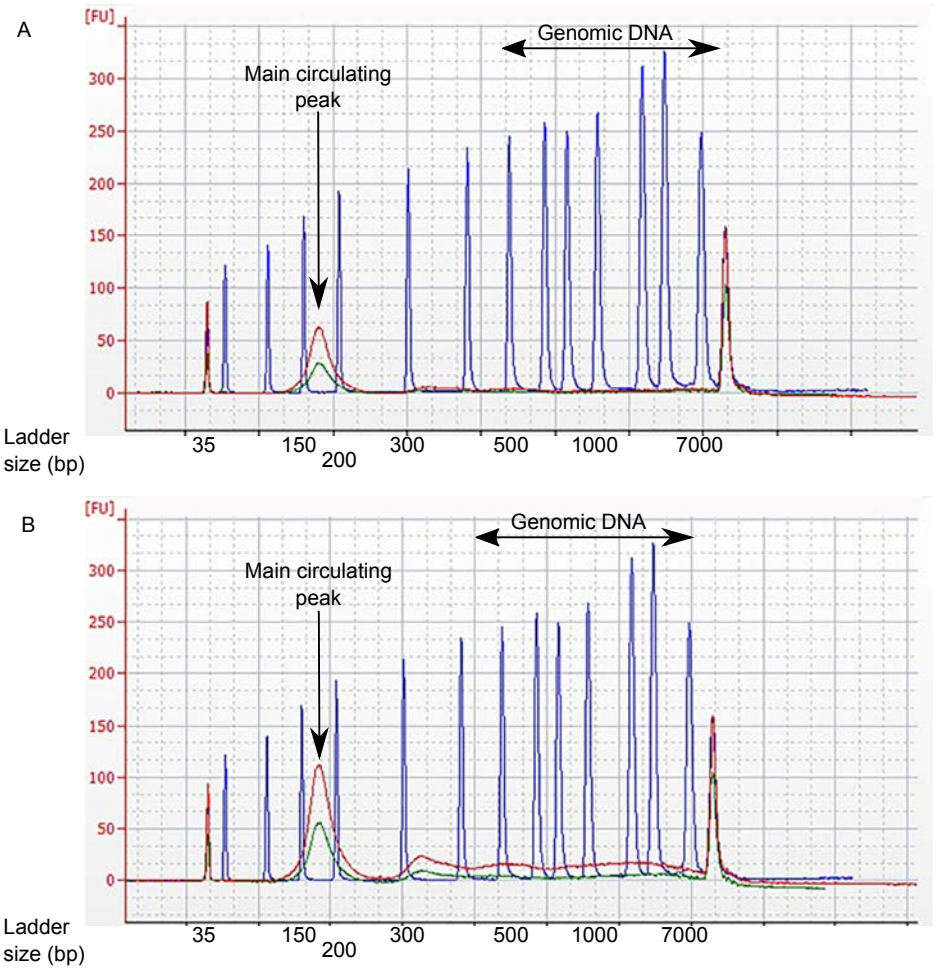

Figure 2 Examples of Agilent™ High Sensitivity DNA Analysis

Analysis on 1 µL of cfDNA isolated from 2 mL (in green) or 4 mL (in red) with the MagMAX™ Cell-Free DNA Isolation Kit. The DNA ladder is in blue with peaks at 35, 40, 100, 150, 200, 300, 400, 500, 600, 700, 1000, 2000, 3000, and 7000 bp. Panel A: sample containing low levels of genomic DNA. Panel B: sample containing increased levels of genomic DNA.

Alternatively, if your yield is high enough, you can use a gel-based smear analysis to estimate cfDNA content in the major peak (100 bp-275 bp).

Protein contamination

We recommend using the Invitrogen™ Qubit™ Protein Assay Kit (Cat. no. Q33212) for absolute quantification.

cfDNA yield measurement

Total cfDNA yield

We recommend using the Invitrogen™ Qubit™ dsDNA HS Assay Kit (Cat. no. Q32855) for total cfDNA yield quantification. This assay is specifically designed for use with the Qubit™ 2.0 Fluorometer (Cat. no. Q32866), but can be used with any fluorometer or fluorescence plate reader.

The assay is highly selective for dsDNA over RNA and is designed to be accurate for initial sample concentrations between 10 pg/µL and 100 ng/µL. It will, however, underestimate the yield of the shorter cfDNA.

Human cfDNA yield

We recommend using the Applied Biosystems™ Quantifiler™ Human DNA Quantification Kit (Cat. no. 4387746) for human cfDNA yield quantification. This assay has been widely used for human gDNA quantification for forensic samples where DNA is often highly degraded. It consists of a real-time TaqMan® assay targeting the single-copy human telomerase reverse transcriptase gene with an amplicon size of 62 bp. A single-copy gene gives more accurate quantification than a multiple-copy gene such as 18S. A single-copy gene assay has the same amplification efficiency for fragmented and non-fragmented gDNA. The short amplicon ensures better detection of highly fragmented DNA. This assay will, however, underestimate the yield of the shorter cfDNA.

D

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

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  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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