applied biosystems

MagMAX™ Cell-Free DNA Isolation Kit

USER GUIDE

Isolation of cfDNA from plasma, serum, and raw saliva samples

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ThermoFisher SCIENTIFIC

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

Revision history: MAN0014327 F00 (English)

Revision	Date	Description
F00	19 March 2024	• Purification procedures were added for saliva samples. • Support was added for the KingFisher™ Apex instrument.
E.0	20 June 2019	The magnetic particle processor was changed from 96DW to 24DW.
D.0	14 July 2017	A product number and the manufacturing address were updated.
C.0	4 August 2016	The guidelines for handling 10-mL samples were updated.
B.0	25 March 2016	A shaking step to bind cfDNA to the beads was added to the manual isolation protocol.
A.0	2 September 2015	New document for the MagMAX™ Cell-Free DNA Isolation Kit.

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

Contents

1 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 raw saliva samples. The kit uses Dynabeads™ MyOne™ SILANE technology and extraction chemistry, ensuring reproducible recovery of high-quality cell-free DNA (cfDNA) that is appropriate for a broad range of applications, including sequencing, genotyping, and qPCR. Stabilized saliva has not been validated with this kit chemistry.

This guide describes isolation of cfDNA from plasma, serum, and saliva samples. Four optimized methods are available:

Table 1 Optimized methods for cfDNA purification using the MagMAX™ Cell-Free DNA Isolation Kit

Sample type	Method
Plasma Serum Saliva	KingFisher™ Apex instrument with 24 Combi head (24-well deep-well setting)
	KingFisher™ Flex instrument with 24 deep-well head (24-well deep-well setting)
Plasma Serum	KingFisher™ Duo Prime instrument (6-well deep-well setting)
	Manual sample processing—8 separate plasma or serum samples can be processed in <1 hour

Alternative purification methods are also available for the following downstream applications.

- **For downstream applications using Ion AmpliSeq™ technology—**See Appendix B, "Alternate method for isolation of higher-concentration cfDNA (plasma and serum)".
- **For downstream applications using Ion Torrent™ technology—**See Appendix B, "Alternate method for isolation of higher-concentration cfDNA (plasma and serum)".
- **For downstream applications that require more concentrated cfDNA in smaller elution volumes—**See Appendix B, "Alternate method for isolation of higher-concentration cfDNA (plasma and serum)".
- **For smaller volumes of plasma and serum samples—**See the *MagMAX™ Cell-Free DNA Isolation Kit User Bulletin (small volumes of plasma and serum samples)* (Pub. no. MAN0015629).

Contents and storage

Table 2 MagMAX™ Cell-Free DNA Isolation Kit (Cat. No. A29319)

Item	Amount[1]	Storage
MagMAX™ Cell-Free DNA Magnetic Beads	1.5 mL	2–8°C Do not freeze.
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] Larger-volume stand-alone reagents can also be ordered separately (see "Related products" on page 42).

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Table 3 Materials required for cfDNA isolation (all methods)

Item	Source
Equipment	
Thermo Scientific™ Compact Digital Microplate Shaker	88880023
Adjustable micropipettors	**MLS**
Multichannel 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**
SDS, 20% Solution (required for Proteinase K treatment)	**MLS**
Proteinase K Solution (20 mg/mL) (required for Proteinase K treatment)	AM2548

Table 4 Additional materials required for automated cfDNA isolation

Item	Source
Instrument, one of the following, depending on sample volume:	
KingFisher™ Apex with 24 Combi head	5400940
KingFisher™ Flex with 24 deep-well head	5400640
KingFisher™ Duo Prime	5400110
Plates and combs	
KingFisher™ 24 deep-well plates, sterile[1]	95040480
Tip comb, compatible with the instrument used:	
KingFisher™ 24 deep-well tip comb and plate, for use with KingFisher™ Apex and KingFisher™ Flex instruments	97002610
KingFisher™ Duo Prime 6-tip comb	97003510
Consumables	
MicroAmp™ Clear Adhesive Film	4306311

[1] Compatible with the KingFisher™ Apex, KingFisher™ Flex, and KingFisher™ Duo Prime instruments

Table 5 Additional materials required for manual cfDNA isolation

Item	Source
Equipment	
Fisherbrand™ Analog Vortex Mixer, or equivalent vortex mixer	02-215-365
DynaMag™–50 Magnet	12302D
DynaMag™-2 Magnet	12321D
Eppendorf™ Thermomixer™ Temperature Control Device (required for Proteinase K treatment)	05-412-503
Reagent	
TAE (10X), RNase-free (required for the alternate method for isolation of higher-concentration cfDNA)	AM9869

2 Methods

Note:

- **For downstream applications using Ion AmpliSeq™ technology:** refer to the protocols described in Appendix B, “Alternate method for isolation of higher-concentration cfDNA (plasma and serum)”.
- **For downstream applications using Ion Torrent™ technology:** refer to the protocols described in Appendix B, “Alternate method for isolation of higher-concentration cfDNA (plasma and serum)”.

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 the shaker and test speeds using the 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 sufficient 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, however, it will not affect the efficiency of the extraction.

- **IMPORTANT!** MagMAX™ Cell-Free DNA Wash Solution and MagMAX™ Cell-Free DNA Lysis/Binding Solution can develop inert white or brown particulates that float in the solutions. Such particulates are not a cause for concern and will not affect kit performance.

- 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.

Guidelines for whole blood collection, and plasma or serum sample generation

- The kit is optimized for plasma samples generated from whole blood collected in K_2EDTA tubes and Streck Cell-Free DNA BCT devices. Whole blood collected in Serum Separator tubes (SST) to generate serum samples has been tested.
- For samples collected in a preservative- or fixative-containing tube, perform a Proteinase K and SDS digestion step before starting the isolation procedure. Plasma samples collected in Streck Cell-Free DNA BCT devices, then treated with Proteinase K show increased cfDNA yields of up to 50%.

Note: Whole blood collected in Streck Cell-Free DNA BCT devices with formaldehyde-free preservative is stable for up to 14 days.

Guidelines for saliva sample collection

- Ensure that there was no eating, drinking, smoking, chewing tobacco, chewing gum, brushing teeth, or use of mouthwash for 10–30 minutes before saliva sample collection.
- Ten to 30 minutes before saliva collection, rinse the mouth with water by swishing water for 10 seconds then spitting the water out to rid mouth of debris.
- Wash hands and remove any lip cosmetics, such as lipstick, lip gloss, or lip balm before saliva collection.
- Use the passive drool technique to pool saliva in the mouth, then drool into a collection device. If needed, stimulate saliva production by gently massaging cheeks or thinking about a favorite food. Do not forcibly spit into the collection device or introduce any bubbles during the saliva collection.
- Ensure only saliva is collected by using the passive drool technique, with no coughing or collection of phlegm.

Set up the instrument (automated methods)

1. On the MagMAX™ Cell-Free DNA Isolation Kit product page (at **thermofisher.com**, search by catalog number), download the appropriate protocol for the instrument, according to the sample type.

Table 6 Instrument protocols

Instrument	Total plasma or serum volume		Total saliva volume
	2 mL	4 mL or 5 mL	2 mL
KingFisher™ Apex with 24 Combi head	**MagMAX_cfDNA_2mL_v1.kfx**	**MagMAX_cfDNA_4mL_V2.kfx**	**MagMAX_cfDNA_2mL_v1.kfx**
KingFisher™ Flex with 24 deep-well head	**MagMAX cfDNA-2mL-Flex.bdz**	**MagMAX cfDNA-4mL-Flex.bdz**	**MagMAX cfDNA-2mL-Flex.bdz**
KingFisher™ Duo Prime	**MagMAX cfDNA-2mL-Duo.bdz**	**MagMAX cfDNA-4mL-Duo.bdz**	—

2. See the instructions from the manufacturer to install the protocol on the instrument.

Perform cfDNA purification using the KingFisher™ Apex instrument with 24 Combi head (plasma and serum)

Prepare cell-free samples

1. Centrifuge the blood samples at 2,000 × *g* for 10 minutes at 4°C.
2. Transfer the sample to a new centrifuge tube.
3. Centrifuge the samples at 16,000 × *g* for 10 minutes at 4°C.

Note: Alternatively, the samples can be centrifuged at 6,000 × *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 samples were collected in a tube containing a preservative, such as a Streck Cell-Free DNA BCT device. Otherwise, proceed directly to the next section.

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

Reagents	Sample volume[1]		
	1 mL	2 mL	4 mL
Proteinase K, 20 mg/mL	15 µL	30 µL	60 µL
Plasma or serum 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, then incubate at 60°C for 20 minutes on the Eppendorf™ Thermomixer™ device.

3. At the end of the 20-minute incubation, place the tubes containing the sample 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.

IMPORTANT! Make sure to use the volumes described in the following tables. Do not use the volumes displayed by the instrument.

Table 7 KingFisher™ Apex with 24 Combi head: Plate setup for 2 mL of plasma or serum

Plate ID	Plate position[1]	Reagent	Volume per well
Sample Plate 1	2	MagMAX™ Cell-Free DNA Lysis/Binding Solution	2.5 mL
		MagMAX™ Cell-Free DNA Magnetic Beads	30 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	1	Place a deep-well comb in a plate.	

[1] Position on the instrument

Table 8 KingFisher™ Apex with 24 Combi head: Plate setup for 4 mL or 5 mL of plasma or serum

Plate ID	Plate position[1]	Reagent	Volume per well	
			4 mL of sample	5 mL of sample
Sample Plate 1	2	MagMAX™ Cell-Free DNA Lysis/Binding Solution	2.5 mL	3 mL
		MagMAX™ Cell-Free DNA Magnetic Beads	60 µL	74 µL
Sample Plate 2	3	MagMAX™ Cell-Free DNA Lysis/Binding Solution	2.5 mL	3 mL
Wash Plate 1	4	MagMAX™ Cell-Free DNA Wash Solution	1 mL	
Wash Plate 2	5	MagMAX™ Cell-Free DNA Wash Solution	1 mL	
Wash Plate 3	6	80% Ethanol	2 mL	
Wash Plate 4	7	80% Ethanol	500 µL	
Elution Plate	8	MagMAX™ Cell-Free DNA Elution Solution	50–100 µL	
Tip Comb	1	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 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, then select the protocol on the instrument according to the following table.

For a total plasma/serum volume of:	Protocol
2 mL	**MagMAX_cfDNA_2mL_v1.kfx**
4 mL	**MagMAX_cfDNA_4mL_V2.kfx**
5 mL	**MagMAX_cfDNA_4mL_V2.kfx**

2. Start the run and load the prepared processing plates in their positions when prompted by the instrument (see Table 9 or Table 10).

3. At the end of the run (about 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.

Perform cfDNA purification using the KingFisher™ Flex instrument with 24 deep-well head (plasma and serum)

Prepare cell-free samples

1. Centrifuge the blood samples at 2,000 × *g* for 10 minutes at 4°C.

2. Transfer the sample to a new centrifuge tube.

3. Centrifuge the samples at 16,000 × *g* for 10 minutes at 4°C.

Note: Alternatively, the samples can be centrifuged at 6,000 × *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 samples were collected in a tube containing a preservative, such as a Streck Cell-Free DNA BCT device. Otherwise, proceed directly to the next section.

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

Reagents	Sample volume[1]		
	1 mL	2 mL	4 mL
Proteinase K, 20 mg/mL	15 µL	30 µL	60 µL
Plasma or serum 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, then incubate at 60°C for 20 minutes on the Eppendorf™ Thermomixer™ device.

3. At the end of the 20-minute incubation, place the tubes containing the sample 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.

   IMPORTANT! Make sure to use the volumes described in the following tables. Do not use the volumes displayed by the instrument.

Table 9 KingFisher™ Flex instrument: Plate setup for 2 mL of plasma or serum

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
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 10 KingFisher™ Flex instrument: Plate setup for 4 mL or 5 mL of plasma or serum

Plate ID	Plate position[1]	Reagent	Volume per well	
			4 mL of sample	5 mL of sample
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 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, then select the protocol on the instrument according to the following table.

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

2. Start the run and load the prepared processing plates in their positions when prompted by the instrument (see Table 9 or Table 10).

3. At the end of the run (about 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.

Perform cfDNA purification using the KingFisher™ Duo Prime instrument (plasma and serum)

Prepare cell-free samples

1. Centrifuge the blood samples at 2,000 × *g* for 10 minutes at 4°C.
2. Transfer the sample to a new centrifuge tube.
3. Centrifuge the samples at 16,000 × *g* for 10 minutes at 4°C.

Note: Alternatively, the samples can be centrifuged at 6,000 × *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 samples were collected in a tube containing a preservative, such as a Streck Cell-Free DNA BCT device. Otherwise, proceed directly to the next section.

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

Reagents	Sample volume[1]		
	1 mL	2 mL	4 mL
Proteinase K, 20 mg/mL	15 µL	30 µL	60 µL
Plasma or serum 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, then incubate at 60°C for 20 minutes on the Eppendorf™ Thermomixer™ device.
3. At the end of the 20-minute incubation, place the tubes containing the sample 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.

   IMPORTANT! Make sure to use the volumes described in the following tables. Do not use the volumes displayed by the instrument.

Table 11 KingFisher™ Duo Prime: Plate setup

Plate	Row ID	Plate row[1]	Reagent	Volume per well		
				2 mL of sample	4 mL of sample	5 mL of sample
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, then select the protocol on the instrument according to the following table.

For a total plasma/serum volume of:	Protocol
2 mL	**MagMAX cfDNA-2mL-Duo.bdz**
4 mL	**MagMAX cfDNA-4mL-Duo.bdz**
5 mL	**MagMAX cfDNA-4mL-Duo.bdz**

2. Start the run and load the prepared processing plates when prompted by the instrument (see Table 11).

   Plate 2 is loaded before Plate 1.

3. At the end of the run (about 35 minutes after the 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.

Perform cfDNA purification using the manual method (plasma and serum)

Workflow

Prepare cell-free samples (page 20)

↓

Option 1: Lyse the samples (with PK), then bind the cfDNA to the beads (page 21)

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

Option 2: Lyse the samples (without PK), then bind the cfDNA to the beads (page 22)

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Wash with Wash Solution (page 22)

↓

Wash twice with 80% ethanol (page 23)

↓

Elute the cfDNA (page 23)

Prepare cell-free samples

1. Centrifuge the blood samples at 2,000 × *g* for 10 minutes at 4°C.
2. Transfer the sample to a new centrifuge tube.
3. Centrifuge the samples at 16,000 × *g* for 10 minutes at 4°C.

Note: Alternatively, the samples can be centrifuged at 6,000 × *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 samples (with PK), then bind the cfDNA to the beads
Others	Option 2: Lyse the samples (without PK), then bind the cfDNA to the beads

Option 1: Lyse the samples (with PK), then bind the cfDNA to the beads

The Proteinase K treatment is required if samples were collected in a tube containing a preservative, such as a Streck Cell-Free DNA BCT device. Otherwise, proceed directly to the next section.

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

Reagents	Sample volume			
	1 mL	2 mL	4 mL	10 mL
Proteinase K, 20 mg/mL	15 µL	30 µL	60 µL	150 µL
Plasma or serum 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, then incubate at 60°C for 20 minutes on the Eppendorf™ Thermomixer™ device.

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

Reagents	Sample 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 Binding Solution/Bead Mix	1.265 mL	2.53 mL	5.06 mL	12.65 mL

4. At the end of the 20-minute incubation, place the tubes containing the sample on ice for 5 minutes.

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

Reagents	Sample volume			
	1 mL	2 mL	4 mL	10 mL
Binding Solution/Bead Mix	1.265 mL	2.53 mL	5.06 mL	12.65 mL

6. Thoroughly mix the sample and the Binding Solution/Bead 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 greater) 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, then remove the remaining supernatant with a pipette.

11. Proceed directly to "Wash with Wash Solution" on page 22.

Option 2: Lyse the samples (without PK), then bind the cfDNA to the beads

1. Prepare the Binding Solution/Bead Mix according to the following table, then mix thoroughly.

Reagents	Sample 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 Binding Solution/Bead Mix	1.265 mL	2.53 mL	5.06 mL	12.65 mL

2. Add the appropriate volume of sample.

3. Thoroughly mix the sample and the Binding Solution/Bead 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 greater) 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, then remove the remaining 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, then store:

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

Perform cfDNA purification using the KingFisher™ Apex instrument with 24 Combi head (saliva)

Prepare cell-free samples

1. Centrifuge saliva samples at 16,000 × *g* for 10 minutes at 4°C.

2. Carefully transfer the sample to a new centrifuge tube.

Treat the samples with Proteinase K

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

Reagents	Volume
Proteinase K, 20 mg/mL	30 µL
Saliva sample	2 mL
SDS, 20% Solution[1]	100 µL
Total volume	2.13 mL

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

2. Mix well, then incubate at 60°C for 20 minutes on the Eppendorf™ Thermomixer™ device.
   During the incubation, set up the processing plates outside the instrument. See “Set up the processing plates” on page 25.

3. At the end of the 20-minute incubation, place the tubes containing the samples on ice for 5 minutes.

Set up the processing plates

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

Table 12 KingFisher™ Flex instrument: Plate setup for 2 mL of saliva

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

[1] Position on the instrument

2. Gently shake Sample Plate 1 to mix the reagents.

3. Add 2 mL of the digested sample to the wells of Sample Plate 1.

Bind, wash, then elute the cfDNA

1. Ensure that the instrument is set up for processing with the deep-well magnetic head, then select the protocol (**MagMAX_cfDNA_2mL_v1.kfx**) on the instrument.

2. Start the run, then load the prepared processing plates in their positions when prompted by the instrument.

3. At the end of the run (about 35 minutes after the initial start), remove the Elution Plate from the instrument, then 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 or at –20°C for long-term storage.

Perform cfDNA purification using the KingFisher™ Flex instrument with 24 deep-well head (saliva)

Prepare cell-free samples

1. Centrifuge saliva samples at 16,000 × *g* for 10 minutes at 4°C.
2. Carefully transfer the sample to a new centrifuge tube.

Treat the samples with Proteinase K

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

Reagents	Volume
Proteinase K, 20 mg/mL	30 µL
Saliva sample	2 mL
SDS, 20% Solution[1]	100 µL
Total volume	2.13 mL

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

2. Mix well, then incubate at 60°C for 20 minutes on the Eppendorf™ Thermomixer™ device.
   During the incubation, set up the processing plates outside the instrument. See “Set up the processing plates” on page 26.
3. At the end of the 20-minute incubation, place the tubes containing the samples on ice for 5 minutes.

Set up the processing plates

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

Table 13 KingFisher™ Flex instrument: Plate setup for 2 mL of saliva

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

[1] Position on the instrument

2. Gently shake Sample Plate 1 to mix the reagents.

3. Add 2 mL of the digested sample to the wells of Sample Plate 1.

Bind, wash, then elute the cfDNA

1. Ensure that the instrument is set up for processing with the deep-well magnetic head, then select the protocol (**MagMAX cfDNA-2mL-Flex.bdz**) on the instrument.

2. Start the run, then load the prepared processing plates in their positions when prompted by the instrument.

3. At the end of the run (about 35 minutes after the initial start), remove the Elution Plate from the instrument, then 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 or 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 23: 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.
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

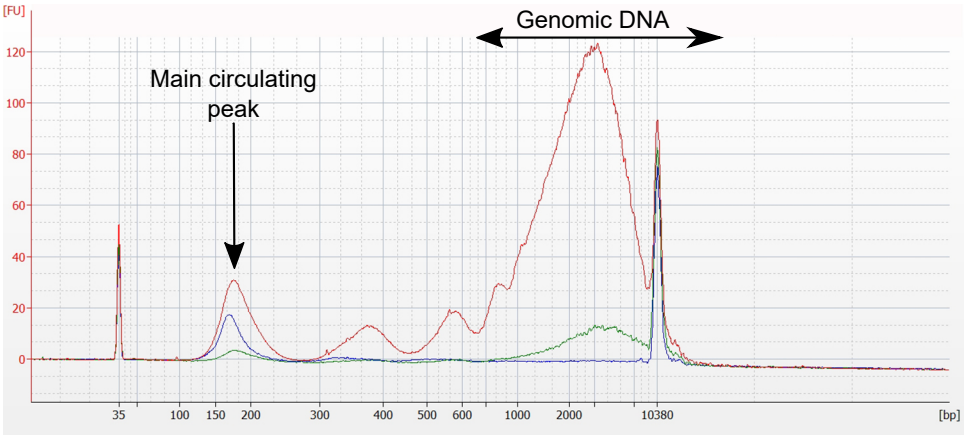

Figure 1 Agilent™ High Sensitivity Analysis on 1 µL of cfDNA isolated from 2 mL of plasma with the MagMAX™ Cell-Free DNA Isolation Kit

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 method for isolation of higher-concentration cfDNA (plasma and serum)

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, however, it will not affect the efficiency of the extraction.
- 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%.

Perform cfDNA purification using the manual method

Prepare cell-free samples

1. Centrifuge the blood samples at 2,000 × *g* for 10 minutes at 4°C.
2. Transfer the sample to a new centrifuge tube.
3. Centrifuge the samples at 16,000 × *g* for 10 minutes at 4°C.

Note: Alternatively, the samples can be centrifuged at 6,000 × *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 32
Others	"Option 2: Lyse the plasma samples (without PK) and bind the cfDNA to the beads" on page 33

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

The Proteinase K treatment is required if samples were collected in a tube containing a preservative, such as a Streck Cell-Free DNA BCT device. Otherwise, proceed directly to the next section.

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

Reagents	Sample volume			
	1 mL	2 mL	4 mL	10 mL
Proteinase K, 20 mg/mL	15 µL	30 µL	60 µL	150 µL
Plasma or serum 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, then incubate at 60°C for 20 minutes on the Eppendorf™ Thermomixer™ device.

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

Reagents	Sample 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 Binding Solution/Bead Mix	1.265 mL	2.53 mL	5.06 mL	12.65 mL

4. At the end of the 20-minute incubation, place the tubes containing the sample on ice for 5 minutes.

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

Reagents	Sample volume			
	1 mL	2 mL	4 mL	10 mL
Binding Solution/Bead Mix	1.265 mL	2.53 mL	5.06 mL	12.65 mL

6. Thoroughly mix the sample and the Binding Solution/Bead 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 greater) 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, then remove the remaining supernatant with a pipette.

11. Proceed directly to step 1 of "Wash with Wash Solution and 80% ethanol" on page 34.

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

1. Prepare the Binding Solution/Bead Mix according to the following table, then mix thoroughly.

Reagents	Sample 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 Binding Solution/Bead Mix	1.265 mL	2.53 mL	5.06 mL	12.65 mL

2. Add the appropriate volume of sample.

3. Thoroughly mix the sample and the Binding Solution/Bead 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 greater) 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, then remove the remaining 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, then 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.

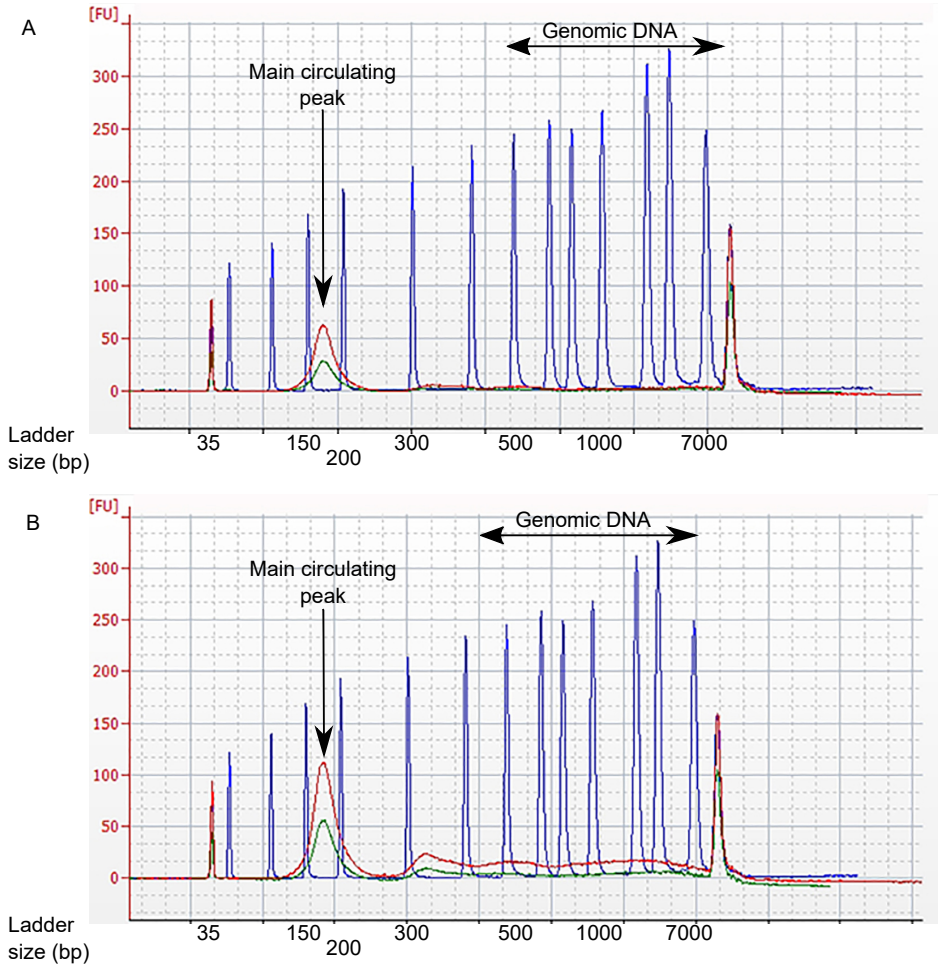

Figure 2 Examples of Agilent™ High Sensitivity DNA Analysis Analysis on 1 µL of cfDNA isolated from 2 mL (in green) or 4 mL (in red) with the MagMAX™ Cell-Free DNA Isolation Kit. The DNA ladder is in blue with peaks at 35, 40, 100, 150, 200, 300, 400, 500, 600, 700, 1000, 2000, 3000, and 7000 bp. Panel 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, and so on). To obtain SDSs, visit **thermofisher.com/support**.

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 sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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 needed) 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.

AVERTISSEMENT ! **PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES.** Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

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)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

Documentation and support

Related products

Item	Amount	Cat. No.
Dynabeads™ MyOne™ SILANE	5 mL	37002D
MagMAX™ Cell-Free DNA Lysis/Binding Solution	425 mL	A33600
MagMAX™ Cell-Free DNA Wash Solution	350 mL	A33601
MagMAX™ Cell-Free DNA Elution Solution	20 mL	A33602

Customer and technical support

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- Worldwide contact telephone numbers
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  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.