MagMAX™ Cell-Free DNA Isolation Kit

Isolation of cfDNA from urine samples

Catalog Number A29319

Pub. No. MAN0015628 Rev. C00

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

Product description

The Applied Biosystems™ MagMAX™ Cell-Free DNA Isolation Kit is designed for isolation of circulating DNA from human urine 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.

This guide describes manual and automated isolation of cfDNA from urine samples.

Contents and storage

The MagMAX™ Cell-Free DNA Isolation Kit provides reagents sufficient to process 60 (1-mL), 30 (2-mL), 20 (4-mL), or 9 (10-mL) urine samples.

Table 1 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 9).

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 2 Materials required for automated cfDNA isolation

Item	Source
Instrument, one of the following:	
KingFisher™ Flex with 96 deep-well head	5400630
KingFisher™ Flex with 24 deep-well head	5400640
KingFisher™ Apex with 96 deep–well head	5400930
KingFisher™ Apex with 24 Combi head	5400940

Item	Source
Plates and tip combs for the KingFisher™ Flex instrument:	
KingFisher™ 96 Deep-Well Plates	95040450
KingFisher™ 24 deep-well plates	95040470
KingFisher™ 96 tip comb for deep-well magnets	97002534
KingFisher™ 24 deep-well tip comb and plate	97002610

Item	Source
Plates and tip combs for the KingFisher™ Apex instrument:	
KingFisher™ 96 Deep-Well Plates, barcoded	95040450B
KingFisher™ 24 deep-well plates, barcoded	95040470B
KingFisher™ 96 tip comb for deep-well magnets, barcoded	97002534B
KingFisher™ 24 deep-well tip comb and plate, barcoded	97002610B
Consumables	
MicroAmp™ Clear Adhesive Film	4306311

Table 3 Materials required for manual cfDNA isolation

Item	Source
Equipment	
Thermo Scientific™ Compact Digital Microplate Shaker	88880023
Vortex mixer	MLS
Adjustable micropipettors	MLS
Multichannel micropipettors	MLS
Refrigerated centrifuge, 4°C	MLS
High-speed centrifuge	MLS
DynaMag™–50 Magnet	12302D
DynaMag™-2 Magnet	12321D
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
100% isopropanol	MLS
TAE (10X), RNase-free	AM9869
Nuclease-free water (not DEPC-treated)	AM9932

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 isolation.
- **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 thoroughly before use.
- If you use a titer plate shaker other than the Thermo Scientific™ Compact Digital Microplate Shaker, ensure that the plate fits securely on the shaker and test speeds using the specific set-up and volumes. Ideal speeds 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. Secure 10– or 50–mL screw-cap conical tubes to the Thermo Scientific™ Compact Digital Microplate Shaker, then shake at speed 7 for the binding step.
- When performing isolation using a KingFisher™ instrument, label the processing plates according to the script.
- 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.
- Use fresh urine stored at 4°C, then centrifuge to cell-free status within 24 hours of collection to avoid increases in genomic DNA (gDNA) and microbial growth. Do not use frozen urine.
  Cell-free urine can be stored at 4°C for up to 3 days without degradation of the major cell-free peak or an increase in gDNA.

Before you begin

For each sample, prepare 300 µL of 0.1X TAE by adding 3 µL of 10X TAE Buffer to 297 µL of Nuclease-Free Water.

Perform cfDNA purification using the KingFisher™ Flex instrument (urine: 10 mL)

1 Set up the instrument

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

Table 4 Programs for the KingFisher™ Flex instrument

Application	Total urine volume	Program
Concentrate cfDNA	10 mL	**MagMAX_cfDNA_Flex_Urine_10mL.bdz**
Rebind cfDNA to magnetic beads	10 mL	**MagMAX_cfDNA_Flex_Urine_Rebind.bdz**

2. Ensure that the instrument is set up with the appropriate magnetic head and heating block for the application.

Table 5 Set-up for the KingFisher™ Flex instrument

Application	Magnetic head	Heating block
Concentrate cfDNA	24-well	24-well
Rebind cfDNA to magnetic beads	96-well	96-well

2 Prepare cell-free urine samples

1. Centrifuge the urine samples at 16,000 × *g* for 10 minutes at 4°C.
2. Transfer the cell-free supernatant to a new centrifuge tube.

The supernatant is ready for immediate use. Alternatively, store the supernatant at 4°C until you are ready to add the samples to the Sample Plate.

3 Prepare Lysis/Binding Solution

Combine the following components in a tube, in the order indicated, for the required number of samples, plus 10% overage (recommended). Vortex thoroughly after adding each component.

Table 6 Preparation of Lysis/Binding Solution

Component	Volume per 10-mL urine sample
MagMAX™ Cell-Free DNA Lysis/Binding Solution	12.5 mL
MagMAX™ Cell-Free DNA Magnetic Beads	150 µL
100% isopropanol	5 mL
Total Lysis/Binding Solution	**17.65 mL**

4 Set up the processing plates for cfDNA concentration

1. Set up the processing plates, according to the following table.

Table 7 Plate setup for cfDNA concentration: KingFisher™ Flex instrument

Plate ID	Plate position[1]	Reagent	Volume per well
Sample Plate 1	1	Prepared Lysis/Binding Solution[2]	3.5 mL
Sample Plate 2	2	Prepared Lysis/Binding Solution	3.5 mL
Sample Plate 3	3	Prepared Lysis/Binding Solution	3.5 mL
Sample Plate 4	4	Prepared Lysis/Binding Solution	3.5 mL
Sample Plate 5	5	Prepared Lysis/Binding Solution	3.5 mL
Wash I Plate Conc	6	MagMAX™ Cell-Free DNA Wash Solution	1 mL
Wash II Plate Conc	7	80% ethanol	1 mL
Tip Comb		Place a 24 deep-well tip comb in a plate	
Elution Plate Conc	8	MagMAX™ Cell-Free DNA Elution Solution	300 µL

[1] Position on the instrument.
[2] Contains MagMAX™ Cell-Free DNA Lysis/Binding Solution + MagMAX™ Cell-Free DNA Magnetic Beads + 100% isopropanol.

2. Evenly distribute the 10-mL urine sample (add 2 mL per well) to identical well locations in Sample Plates 1–5.

5 Process samples on the instrument to concentrate cfDNA

Ensure that the instrument is set up with the appropriate magnetic head and heating block (see "Set up the instrument" on page 3).

1. Select the appropriate program on the instrument (see "Set up the instrument" on page 3).
2. Start the run, then load the prepared plates in the appropriate positions when prompted by the instrument. The run takes about 56 minutes to complete.

   During the run, proceed to set up the processing plates to rebind the cfDNA to the MagMAX™ Cell-Free DNA Magnetic Beads (see Table 8).
3. When the run is complete, remove the elution plate, then transfer the concentrated cfDNA to a new 96-well deep-well plate (Sample Plate Rebi).

6 Set up the processing plates to rebind cfDNA to the magnetic beads

Set up the processing plates, according to the following table.

IMPORTANT! Do not use the volumes displayed on the instrument.

Table 8 Plate setup to rebind cfDNA: KingFisher™ Flex instrument

Plate ID	Plate position[1]	Reagent	Volume per well
Sample Plate Rebi	1	MagMAX™ Cell-Free DNA Lysis/Binding Solution	375 µL
		MagMAX™ Cell-Free DNA Magnetic Beads	10 µL
		100% isopropanol	150 µL
Wash I Plate Rebi	2	MagMAX™ Cell-Free DNA Wash Solution	1 mL
Wash II Plate 1 Rebi	3	80% ethanol	1 mL
Wash II Plate 2 Rebi	4	80% ethanol	1 mL
Elution Plate Rebi	5	MagMAX™ Cell-Free DNA Elution Solution	30 µL
Tip Plate	6	Place a 96 deep-well tip comb in a plate	

[1] Position on the instrument.

7 Process samples on the instrument

Ensure that the instrument is set up with the appropriate magnetic head and heating block (see "Set up the instrument" on page 3).

1. Select the appropriate program on the instrument (see "Set up the instrument" on page 3).
2. Start the run, then load the prepared plates in the appropriate positions when prompted by the instrument. The run takes about 27 minutes to complete.
3. When the run is complete, remove the elution plate, then immediately cover with adhesive film.

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

Store the sealed elution plate containing the purified cfDNA on ice for immediate use (up to 24 hours), or at –20°C for long-term storage.

Perform cfDNA purification using the KingFisher™ Apex instrument (urine: 10 mL)

1 Set up the instrument

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

Table 9 Programs for the KingFisher™ Apex instrument

Application	Total urine volume	Program
Concentrate cfDNA	10 mL	**MagMAX_cfDNA_Apex_Urine_10mL.bdz**
Rebind cfDNA to magnetic beads	10 mL	**MagMAX_cfDNA_Apex_Urine_Rebind.bdz**

2. Ensure that the instrument is set up with the appropriate magnetic head and heating block for the application.

Table 10 Set-up for the KingFisher™ Apex instrument

Application	Magnetic head	Heating block
Concentrate cfDNA	24-well	24-well
Rebind cfDNA to magnetic beads	96-well	96-well

2 Prepare cell-free urine samples

1. Centrifuge the urine samples at 16,000 × *g* for 10 minutes at 4°C.
2. Transfer the cell-free supernatant to a new centrifuge tube.

The supernatant is ready for immediate use. Alternatively, store the supernatant at 4°C until you are ready to add the samples to the Sample Plate.

3 Prepare Lysis/Binding Solution

Combine the following components in a tube, in the order indicated, for the required number of samples, plus 10% overage (recommended). Vortex thoroughly after adding each component.

Table 11 Preparation of Lysis/Binding Solution

Component	Volume per 10-mL urine sample
MagMAX™ Cell-Free DNA Lysis/Binding Solution	12.5 mL
MagMAX™ Cell-Free DNA Magnetic Beads	150 µL
100% isopropanol	5 mL
Total Lysis/Binding Solution	**17.65 mL**

4 Set up the processing plates for cfDNA concentration

1. Set up the processing plates, according to the following table.

Table 12 Plate setup for cfDNA concentration: KingFisher™ Apex instrument

Plate ID	Plate position[1]	Reagent	Volume per well
Sample Plate 1	2	Prepared Lysis/Binding Solution[2]	3.5 mL
Sample Plate 2	3	Prepared Lysis/Binding Solution	3.5 mL
Sample Plate 3	4	Prepared Lysis/Binding Solution	3.5 mL
Sample Plate 4	5	Prepared Lysis/Binding Solution	3.5 mL
Sample Plate 5	6	Prepared Lysis/Binding Solution	3.5 mL
Wash I Plate Conc	7	MagMAX™ Cell-Free DNA Wash Solution	1 mL
Wash II Plate Conc	1	80% ethanol	1 mL
Tip Comb		Place a 24 deep-well tip comb in a plate	
Elution Plate Conc	8	MagMAX™ Cell-Free DNA Elution Solution	300 µL

[1] Position on the instrument.
[2] Contains MagMAX™ Cell-Free DNA Lysis/Binding Solution + MagMAX™ Cell-Free DNA Magnetic Beads + 100% isopropanol.

2. Evenly distribute the 10-mL urine sample (add 2 mL per well) to identical well locations in Sample Plates 1–5.

5 Process samples on the instrument to concentrate cfDNA

Ensure that the instrument is set up with the appropriate magnetic head and heating block (see "Set up the instrument" on page 5).

1. Select the appropriate program on the instrument (see "Set up the instrument" on page 5).
2. Start the run, then load the prepared plates in the appropriate positions when prompted by the instrument. The run takes about 56 minutes to complete.

   During the run, proceed to set up the processing plates to rebind the cfDNA to the MagMAX™ Cell-Free DNA Magnetic Beads (see Table 13).
3. When the run is complete, remove the elution plate, then transfer the concentrated cfDNA to a new 96-well deep-well plate (Sample Plate Rebi).

6 Set up the processing plates to rebind cfDNA to the magnetic beads

Set up the processing plates, according to the following table.

IMPORTANT! Do not use the volumes displayed on the instrument.

Table 13 Plate setup to rebind cfDNA: KingFisher™ Apex instrument

Plate ID	Plate position[1]	Reagent	Volume per well
Sample Plate Rebi	2	MagMAX™ Cell-Free DNA Lysis/Binding Solution	375 µL
		MagMAX™ Cell-Free DNA Magnetic Beads	10 µL
		100% isopropanol	150 µL
Wash I Plate Rebi	3	MagMAX™ Cell-Free DNA Wash Solution	1 mL
Wash II Plate 1 Rebi	4	80% ethanol	1 mL
Wash II Plate 2 Rebi	5	80% ethanol	1 mL
Elution Plate Rebi	6	MagMAX™ Cell-Free DNA Elution Solution	30 µL
Tip Plate	1	Place a 96 deep-well tip comb in a plate	

[1] Position on the instrument.

7 Process samples on the instrument

Ensure that the instrument is set up with the appropriate magnetic head and heating block (see "Set up the instrument" on page 5).

1. Select the appropriate program on the instrument (see "Set up the instrument" on page 5).
2. Start the run, then load the prepared plates in the appropriate positions when prompted by the instrument. The run takes about 27 minutes to complete.
3. When the run is complete, remove the elution plate, then immediately cover with adhesive film.

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

Store the sealed elution plate containing the purified cfDNA on ice for immediate use (up to 24 hours), or at –20°C for long-term storage.

Perform cfDNA purification using the manual method

1 Prepare cell-free urine samples

1. Centrifuge the urine samples at 16,000 × *g* for 10 minutes at 4°C.
2. Transfer the cell-free supernatant to a new centrifuge tube.

The supernatant is ready for immediate use. Alternatively, store the supernatant at 4°C until you are ready to perform the sample lysis step (next section).

2 Lyse the urine samples and bind the cfDNA to the beads

1. Prepare the Binding Solution/Bead/Isopropanol Mix according to the following table. Vortex thoroughly after adding each component.

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
100% Isopropanol	0.5 mL	1 mL	2 mL	5 mL
Total Binding Solution/Bead/Isopropanol Mix	1.765 mL	3.53 mL	7.06 mL	17.65 mL

2. Add the appropriate volume of urine sample.
3. Thoroughly mix the urine sample and the Binding Solution/Bead/Isopropanol 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.

3 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 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. Remove the tube from the DynaMag™-2 Magnet, add 1 mL of 80% ethanol, then vortex for 30 seconds.
13. Place the tube on the DynaMag™-2 Magnet for 2 minutes, or until the solution clears and the beads are pelleted against the magnets.
14. Remove the supernatant with a 1-mL pipette.
15. Keeping the tube on the DynaMag™-2 Magnet, air dry the beads for 3–5 minutes.
16. 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.

4 Elute the cfDNA, rebind, and wash

1. Add 300 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, 375 µL of MagMAX™ Cell Free DNA Lysis/Binding Solution, and 150 µL of 100% isopropanol to the supernatant, then mix thoroughly.
5. 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.

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

4 *(continued)*

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.

5 **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 5.1–step 5.3 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.

6 **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.

Total cfDNA yield measurement

We recommend using the Invitrogen™ Qubit™ dsDNA HS Assay Kit (Cat. No. Q32854) to quantify total cfDNA yield. This assay is designed for use with the Qubit™ 4 Fluorometer (Cat. No. Q33238) but can be used with any fluorometer or fluorescence plate reader. The Qubit™ dsDNA HS Assay is highly selective for dsDNA over RNA and is accurate for initial sample concentrations between 10 pg/µL and 100 ng/µL. The assay, however, will underestimate yield of shorter cfDNA.

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

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**.

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

Revision history: Pub. No. MAN0015628 C00

Revision	Date	Description
C00	19 March 2024	Procedures for automated isolation of cell-free DNA were added to the document.
B.0	13 February 2018	Input sizes and reordering instructions were added to the document.
A.0	25 March 2016	New document for MagMAX™ Cell-Free DNA Isolation Kit.