User Manual

GenElute™ UltraMag Cell-Free DNA Kit

CFMAG

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Not for use in diagnostic procedures. Not for human or animal consumption.

Introduction

GenElute™ UltraMag Cell-Free DNA Kit provides rapid and efficient purification of circulating free DNA, also known as cfDNA. The kit uses silica-coated magnetic beads to purify cell free DNA from serum and plasma samples of less than 1 mL up to 10 mL. The kit was developed to provide efficient recovery cell-free DNA fragments in the range of 100 bp–500 bp. The recovered DNA is suitable for a wide range of down-stream applications including next generation sequencing, qPCR and bisulfite sequencing.

Product Overview

GenElute™ UltraMag Cell-Free DNA Kit allows for fast and efficient cell-free DNA (cfDNA) isolation from plasma/serum samples. The magnetic bead-based extraction protocol is ideally suited for use with robotic liquid handlers. DNA extracted using this kit is suitable for PCR, qPCR, next generation sequencing (NGS), and other applications. The GenElute™ UltraMag Cell-Free DNA extraction kit allows preparation of DNA fragments from both fresh and frozen plasma samples. Samples from fresh plasma, however, tends to produce higher yields. Plasma from blood collected with Streck® Cell-Free DNA BCT tubes must go through a Proteinase K treatment prior to cell-free DNA isolation to ensure optimal yields. Forgoing Proteinase K treatment may decrease yields by 50%. The kit provides high DNA recovery with a short and scalable magnetic bead-based protocol and without the use of phenol chloroform.

Materials Provided

For 100 mL Kit

Item	Catalog No.	Quantity
Lysis/Binding Buffer	CS222724	1 x 115 mL
Wash Buffer	CS222723	2 x 55 mL
Elution Buffer	CS222772	1 x 6 mL
Magnetic Bead Solution	CS222756	2 x 1.33 mL

For 250 mL Kit

Item	Catalog No.	Quantity
Lysis/Binding Buffer	CS222770	3 x 95 mL
Wash Buffer	CS222723	5 x 55 mL
Elution Buffer	CS222768	1 x 15 mL
Magnetic Bead Solution	CS222756	5 x 1.33 mL

Materials Required (Not supplied)

Reagents

- 100% ethanol (E7023 or equivalent)
- Samples collected in a Streck® Cell-Free DNA BCT tube require:
    - Proteinase K (P2308 or equivalent)
    - 20% SDS, (05030 or equivalent)

Equipment

- Vortex mixer
- Magnetic stand for molecular applications (LSKMAGS08, LSKMAGS15 or equivalent)
- 1.5 mL low binding PCR approved vials
- Reaction tubes 2, 15 or 50 mL
- Water bath or heater block

Storage and Stability

Store all kit components at room temperature; performance guaranteed for 1 year from date of receipt when reagents are stored properly.

Protocol

Prior to Use

The Lysis/Binding and Wash Buffer are shipped as a concentrate. If precipitate is present in either solution, incubate solution at 37 °C for 30 minutes. 100% ethanol must be added to both solutions before the first use. Once ethanol is added, these buffers are stable for one year if stored at 4 °C. Be sure to close the bottle tightly for long term storage.

For 100 mL kit

- Add 23 mL of fresh 100% ethanol to each bottle of Lysis/Binding Buffer and mix by inverting gently.
- Add 51 mL of fresh 100% ethanol to each bottle of Wash Buffer and mix by inverting gently.
- Prepare fresh 80% ethanol solution prior to each extraction.

For 250 mL kit

- Add 19 mL of fresh 100% ethanol to each bottle of Lysis/Binding Buffer and mix by inverting gently.
- Add 51 mL of fresh 100% ethanol to each bottle of Wash Buffer and mix by inverting gently.
- Prepare fresh 80% ethanol solution prior to each extraction.

Before starting the protocol, determine the amount of plasma to be used for extraction and calculate the amount of buffer and beads needed. Any amount from 100 µL to 10 mL of plasma can be used. Scale buffer and bead volumes accordingly using the table below.

Plasma	Lysis/Binding Buffer	Bead Solution	Tube(s) Size
500 µL	750 µL	12.5 µL	2 mL
1 mL	1.25 mL	25 µL	15 mL
5 mL	6.25 mL	125 µL	15 mL or 50 mL*
10 mL	12.5 mL	250 µL	50 mL

Note: Using 50 mL tubes for 5 mL or more of plasma is recommended over 15 mL tubes. While using a 15 mL tube will work it may lead to slightly lower yields.

Proteinase K Treatment

If samples were collected using a Streck® Cell-Free DNA BCT tube(s), Proteinase K treatment is required to ensure optimal yields. If blood was not collected with Streck® Cell-Free DNA BCT tube(s) proceed to the Lysis/Binding step.

Plasma	Proteinase K	20% SDS Solution
500 µL	7.5 µL	25 µL
1 mL	15 µL	50 µL
5 mL	75 µL	250 µL
10 mL	150 µL	500 µL

1. Add the appropriate amount of plasma to an appropriately sized tube(s).
2. Add 15 µL of Proteinase K (20 mg/mL) for every 1 mL of plasma used.
3. Add 50 µL of 20% SDS solution for every 1 mL of plasma used.
4. Mix by inverting gently 5 times.
5. Incubate at 60 °C for 20 minutes.
6. After incubation place tube(s) on ice for 5 minutes to cool tube(s) to room temperature.
7. Once tube(s) are at room temperature proceed to step 2 of the Lysis/Binding section.

Lysis and Binding

1. Add the appropriate amount of plasma to appropriately sized tube(s).
8. Add 1.25 mL of Lysis/Binding Buffer for every 1 mL of plasma used.
9. Add 25 µL of thoroughly mixed Magnetic Bead Solution for every 1 mL of plasma.

   Important: Mix beads well prior to adding by vortexing briefly. There should be no visible sedimentation at the bottom of the solution after mixing. Beads will settle quickly, so be sure to mix the magnetic bead solution after adding to each sample. Failure to do so may result in inconsistent yields.
10. Vortex or shake tube(s) vigorously for 10 minutes at room temperature.

    Note: To obtain high yields, ensure that plasma/buffer solution is mixing vigorously in tube(s). A vortexing mixer with a tube(s)-holder that allows for walk-away mixing will make this easier.
11. Place tube(s) into a magnet stand for 2 to 5 minutes, or until solution clears.
12. While keeping the tube(s) on the magnet stand, remove supernatant. Be careful not to remove magnetic particles.
13. Keep tube(s) on magnet stand for 1 minute and remove residual supernatant.

First Wash

1. Add 1000 µL of Wash Buffer to lysis/binding tube(s).
2. Resuspend beads by vortexing for 10 seconds or pipetting up and down 6 times.
3. Transfer magnetic particle suspension into 1.5 mL microcentrifuge tube(s) on magnet stand.
4. Allow beads to attach to magnet stand for 10-30 seconds, or until solution clears.
5. Pipette supernatant from 1.5 mL tube(s) and use the supernatant to wash the lysis/binding tube(s).
6. Transfer the rest of the magnetic particles in lysis/binding tube(s) to the 1.5 mL tube(s).
7. Keep tube(s) on magnet stand for 10-30 seconds or until solution is clear.
8. Remove as much buffer as possible using a 1000 µL pipette.
9. Tap magnet stand on bench 5 times and remove remaining wash buffer with 200 µL pipette.
10. Transfer tube(s) to non-magnetic rack and add 1000 µL of Wash Buffer.
11. Resuspend beads by vortexing for 20 seconds or pipetting up and down 6 times.
12. Centrifuge tube(s) briefly.

    Note: Centrifuge steps are needed when using vortexing to resuspend beads. Only a brief spin is recommended to remove solution from tube(s) lid.
13. Place tube(s) on magnet stand for 10-30 seconds. Remove as much buffer as possible using a 1000 µL pipette.
14. Tap magnet stand on bench 5 times and remove remaining wash buffer with 200 µL pipette.

Second Wash

1. Transfer tube(s) to non-magnetic rack and add 1000 µL of 80% ethanol.
2. Resuspend beads by vortexing for 20 seconds or pipetting up and down 6 times.
3. Centrifuge tube(s) briefly.
4. Place on magnet stand for 10-30 seconds or until solution clears.
5. Remove as much buffer as possible using a 1000 µL pipette.
6. Tap magnet stand on bench 5 times and remove remaining ethanol with 200 µL pipette.
7. Transfer tube(s) to non-magnetic rack and add 1000 µL of 80% ethanol.
8. Resuspend beads by vortexing for 20 seconds or pipetting up and down 6 times.
9. Centrifuge tube(s) briefly.
10. Place on magnet stand for 10-20 seconds.
11. Remove as much ethanol as possible using a 1000 µL pipette and leave cap open.
12. Tap magnet stand with tube(s) on bench 5 times.
13. Remove remaining ethanol with 200 µL pipette.
14. Leave tube(s) open on magnet stand for two minutes and then tap tube(s) on bench 5 times and remove any remaining ethanol with 20 µL pipette.
15. Allow magnetic particles to dry for an additional 1-3 minutes. Be careful to not over dry or beads may stick to tube(s).

Elution

1. Transfer microcentrifuge tube(s) to non-magnetic rack and add desired volume of Elution Buffer and resuspend beads.

   Important: A minimum of 12.5 µL of Elution Buffer per mL of plasma is recommended to elute DNA to ensure optimal yields.
2. Vortex or shake tube(s) vigorously for 5 minutes.
3. Centrifuge tube(s) briefly.
4. Place tube(s) on magnetic rack for 10 to 30 seconds.
5. Transfer elutes into a new 1.5 mL tube(s).

Important Notes

Quantification: Plasma will yield 1-100 ng of Cell-Free DNA per mL of plasma. Therefore, quantification by absorbance measurement (for example, Nanodrop) may not be sensitive enough to accurately determine yield. Instead, we suggest using Qubit® dsDNA High Sensitivity Assay.

Recommendations for PCR: Due to the highly fragmented nature of the nucleic acids obtained from plasma, care should be taken in the design of primers. Cell-free DNA tends to have a small size (~170 bp). Therefore, PCR primers should be designed to produce amplicons of 150 bp or less. Given the low concentration of cfDNA in plasma taken from healthy individuals, 40 amplification cycles may be needed in some cases.

Troubleshooting

Observation	Potential Problems	Experimental Suggestions
Lower than expected yield	Sample degradation during storage.	Repeated freezing and thawing should be avoided. Minimize time between blood draw and plasma preparation if possible.
	An insufficient amount of DNA Magnetic Beads was added.	Vortex the Magnetic Bead Solution immediately before use.
	Magnetic beads are difficult to resuspend after drying step.	Smaller volumes require less time to dry. Airflow and humidity of the immediate environment may change the optimal bead drying times. Overdried beads will stick to the wall of the plastics and be difficult to resuspend.
	Magnetic Beads are not completely dried.	Beads that have not been completely dried may carry ethanol into the eluate and impact downstream applications.
	The sample contains low levels of cfDNA.	Increase the volume of the starting sample.
	Insufficient mixing of sample with magnetic beads during the binding step.	After adding your sample to the tube containing the DNA Lysis/Binding solution and the magnetic beads, tightly seal the conical tube, and shake for the binding step. Alternatively, mix vigorously using a vortex set to maximum speed for 10 minutes using a vortex tube adapter.
Magnetic Bead carryover	Loose beads are present in the eluate.	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 into another tube.

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