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User Guide

Ultra Pure MagPrep® Cell-Free DNA Isolation Kit

Isolation of cell-free DNA (cfDNA) from biological fluids (**Cat. No. UPMPCFDNA-KT**)

Introduction

Ultra pure DNA-free MagPrep® Cell-Free DNA (cfDNA) Isolation Kit can be utilized for manual or high throughput cfDNA isolation from biological fluids such as plasma, urine, CSF, saliva, etc. Buffers have been designed to maximize the performance of the magnetized silica particles and this rapid and simple protocol describes manual isolation of cfDNA. Buffer components are compatible with automation systems. The user-friendly protocol has no Proteinase K or heating steps, and all items are shipped and stored at ambient temperature.

All kit components (MagPrep® particles and buffers) are tested to be Ultra pure i.e. free of any Nickase, DNase, RNase, and DNA-free (tested by qPCR for 16S-, 18S-RNA genes and plasmid Ori). The DNA-free Ultra pure feature of the kit enables reliable results in downstream applications without the fear of false positives (DNA-free) and false negatives (nuclease-free).

The purified cfDNA is free of any PCR inhibitors (chaotropic agents, ethanol, etc.) and can be used for various downstream applications such as qPCR, ddPCR, NGS, bisulfite-SEQ, etc.

For research and further manufacturing use only.

Principle

MagPrep® magnetic particles are an easy and fast method to isolate nucleic acids. The magnetized silica particles are incubated with a matrix containing cfDNA and MagPrep® cfDNA Binding Buffer. cfDNA binds to the particles and unwanted materials are washed away using a magnet. The isolated cfDNA is then eluted using MagPrep® Elution Buffer. The process can be automated or performed manually in less than 30 minutes and yields high recovery of pure cfDNA without any PCR inhibitors (see **Figure 1**).

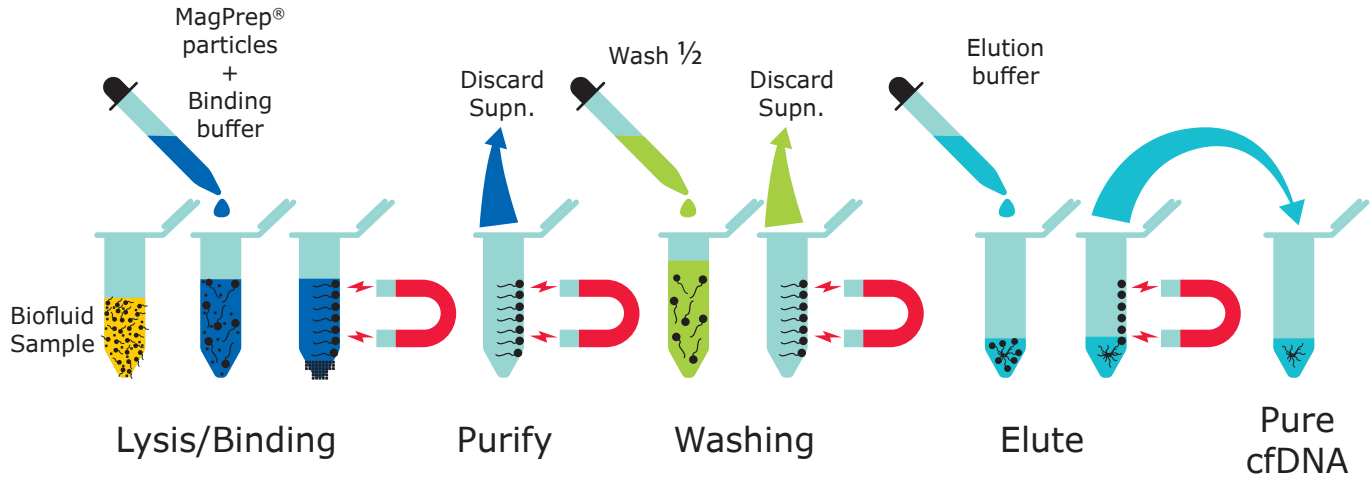

Figure 1: Graphical image of the process to create Ultra Pure cfDNA.

Storage Conditions

Store kit components at ambient temperature.

DO NOT FREEZE.

Reagents Supplied:

100 reactions or one 96-well plate

Ultra pure MagPrep® Cell-Free DNA Isolation kit, **Cat. No. UPMPCFDNA-KT**

Kit Component Name	Cat. No.	Volume	Quantity
Ultra pure MagPrep® cfDNA Binding Buffer	**UPMPCFDBB-KC**	50 mL	1 bottle
Ultra pure MagPrep® Wash Buffer 1	**UPMPWB1-KC**	100 mL	1 bottle
Ultra pure MagPrep® Wash Buffer 2	**UPMPWB2-KC**	100 mL	1 bottle
Ultra pure MagPrep® Elution Buffer	**UPMPEB-KC**	10 mL	1 bottle
Ultra pure MagPrep® Magnetic Particles	**UPMPPART-KC**	2 mL	1 vial

Individual Components available separately:

Component Name	Cat. No.	Volume	Quantity
Ultra pure MagPrep® cfDNA Binding Buffer	**UPMPCFDBB**	250 mL, 500 mL	1 bottle
Ultra pure MagPrep® Wash Buffer 1	**UPMPWB1**	300 mL, 500 mL	1 bottle
Ultra pure MagPrep® Wash Buffer 2	**UPMPWB2**	300 mL, 500 mL	1 bottle
Ultra pure MagPrep® Elution Buffer	**UPMPEB**	100 mL, 250 mL	1 bottle
Ultra pure MagPrep® Magnetic Particles	**UPMPPART**	10 mL, 20 mL	1 vial

Materials Required (not included)

Reagents are needed for downstream applications (e.g. qPCR, ddPCR, NGS, Bisulfite-SEQ, etc.); review your application protocol to determine which reagents are needed.

Instrumentation/Materials

- Magnetic rack
- Rotating mixer
- Adjustable Pipettes with tips capable of delivering 25 µL to 1000 µL
- Polypropylene Microfuge Tubes
- Laboratory Vortex Mixer
- Benchtop microfuge

Safety Precautions

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

Isopropanol and ethanol have been added to some of the buffers and must be treated as flammables. Other ingredients may have low amounts of corrosive materials as well as materials toxic to aquatic life. Dispose of unused contents and waste following international, federal, state, and local regulations.

Technical Guidelines

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. The following notes should be reviewed and understood before the assay is set up.

For research and further manufacturing use only. Not for use in diagnostic procedures.

Do not use beyond the expiration date on the label.

Do not mix or substitute reagents with those from other lots or sources.

The binding buffer is light sensitive. It is packaged in a light protective bottle and must be protected from light if aliquots are taken from the original bottle.

Incomplete washing can adversely affect isolation purity. All washing must be performed with the Wash Buffers provided.

Be aware that nucleic acid can be sensitive to degradation at room temperature. Ensure isolation using this kit is conducted in a timely matter.

After the second wash, ensure complete removal of Wash Buffer 2.

Ensure components are recapped right after each use.

Binding/Lysis buffer may change to a yellowish color. This does not affect performance.

Sample Collection and Storage

Preparation of Plasma Samples:

Plasma collection using EDTA as an anti-coagulant is recommended in cfDNA storage-compatible tubes such as BCT Streck. Centrifuge for 10 minutes at 1000 xg within 30 minutes of blood collection and carefully collect the plasma above the buffy coat avoiding the floating lipids. A second spin at 6000 xg for 30 minutes at 4 °C is recommended for the removal of cellular debris and carryover lipids to avoid genomic DNA contamination into the plasma. Remove the

supernatant and filter through a 0.2 µm filter. Remove plasma and assay immediately or aliquot and store samples at ≤–20 °C.

Avoid multiple (>2) freeze/thaw cycles.

When using frozen samples, it is recommended to thaw the samples completely and mix them well by vortexing and centrifuging before use in the assay to remove particulates.

Fresh centrifuged and filtered plasma gives better recovery.

Nucleic Acid Isolation Procedure

(Applicable to any biological fluid sample)

Manual Protocol

Scale: Protocol is written for 0.5 mL biological fluid (e.g. plasma) as starting material that should be mixed with 0.5 mL of cfDNA binding buffer containing ≈0.75 mg MagPrep® particles for each binding reaction (step 1). The protocol is scalable provided the 1:1 volume ratio is maintained between the biological fluid and cfDNA binding buffer plus MagPrep® particles solution.

Gently shake the buffers before use. Resuspend the MagPrep® particles stock solution thoroughly by vortexing before taking an aliquot.

1. In a 1.5 mL DNase- and RNase-free microfuge tube, add 14 µL (≈0.75 mg) of Ultra pure MagPrep® particles to 500 µL of Ultra pure cfDNA Binding Buffer and mix well by pipetting up-down or vortexing.
2. Add 500 µL of biological fluid sample (e.g. plasma) and mix well by pipetting up and down 10–15x or vortexing intermittently 10 times at a medium speed.
3. Incubate for 5 minutes to allow cfDNA binding (Optional: a rotation system to mix during incubation may be used).
4. Place the tube on a magnetic rack for 1–2 minutes to capture the cfDNA-particle complexes, then discard the supernatant.
5. Remove the tube from the magnetic rack and resuspend the cfDNA/particle complexes in 1 mL of Ultra pure Wash Buffer 1 by vortexing. Use a microfuge to spin and bring all contents down quickly.
6. Return to the magnetic rack for 1–2 minutes, then discard the supernatant.
7. Repeat wash with 1 mL of Ultra pure Wash Buffer 2. Use a microfuge to spin and bring all contents down quickly.
8. Return to the magnetic rack for 1–2 minutes, then discard the supernatant.
9. *Essential*: Do a quick spin on a microfuge to collect remnants of wash buffer sticking to the sides of the tubes and particles and return to the magnetic rack. Remove any remainder supernatant.
10. Dry the tube with the lid open at room temperature for 5 minutes. Dry for longer if pellet still looks wet.
11. After the ~5 minute drying step, remove any visible supernatant.
12. Remove the tube from the magnetic rack and resuspend the particles in 50 µL of Ultra pure Elution Buffer.
13. To elute cfDNA from particles, mix by pipetting 15–20 x or vortexing intermittently 10 times at a medium speed.
14. Let stand for 1–2 minutes. Do a quick spin in a microfuge to bring all contents down.
15. Place the tube on a magnetic rack for 1–2 minutes to separate particles from the suspension.
16. Transfer the supernatant containing the isolated cfDNA solution to a clean DNase- and RNase-free tube.
17. Store at 4 °C for 2 days or at –20 °C for long-term storage.
18. Purified cfDNA can be used for downstream analysis such as qPCR, ddPCR, NGS, ARMS, BEAMing, bisulfite-SEQ, etc.

Troubleshooting Guide

Problem	Probable Cause	Solution
Poor cfDNA recovery	Insufficient magnetic particles were aliquoted from the MagPrep® particles stock solution	Ensure the MagPrep® particles stock solution was mixed thoroughly by vortexing before taking an aliquot.
	Very dilute plasma sample	Increase sample volume and/or increase particle binding incubation time.
	cfDNA binding buffer was skipped and MagPrep® particles were added directly to the plasma sample	Dilute MagPrep® particles in cfDNA binding buffer before adding them to plasma. Binding buffer removes proteins from cfDNA and facilitates DNA binding to particles.
	The primary blood collection tube did not contain EDTA or there was an extended time lapse between sample collection and plasma preparation, samples had multiple freeze-thaw cycles before cfDNA purification	EDTA prevents DNA degradation other anticoagulants may not. It is best to collect samples in EDTA BCT Streck tubes. Freeze rapidly for transport and thaw once, double spin to clarify plasma before rapid cfDNA purification.

Problem	Probable Cause	Solution
Inhibition of PCR	The sample has too much debris	Double spin plasma as mentioned below and filter (0.2 µm) before starting the cfDNA purification. Perform 1–2 additional washes with Wash Buffer 2
	The sample contains residual alcohol from Wash Solution 2	Post removal of Wash Solution 2 supernatant, remove the vial from the magnetic rack and spin briefly in a microfuge. Place back on a magnetic rack for 2 minutes and use a P20 pipette to remove any supernatant left in the vial.
	The sample contains magnetic particles	Avoid disturbing the magnetic particles while removing the supernatant. Angle the pipette so that the tip does not touch the magnetic particles. If particles are still observed in the final eluate they can be removed by another short magnetic separation on the rack.
Particles are not migrating to the magnet	The sample is too viscous	Dilute the sample or increase the time on a magnetic rack to >5 minutes.
Genomic DNA contamination in final cfDNA elution	Genomic DNA was released into biological fluid samples from contaminating cells	Double spin the biological fluid sample (blood, plasma, or urine) at 6000 xg for 30 minutes at 4 °C to remove cells and cellular debris. Filter supernatant through 0.2 µm filter and store at –20 °C until ready for cfDNA purification.

References:

1. Liquid biopsy: a step closer to transform diagnosis, prognosis, and future of cancer treatments. Lone et al. Molecular Cancer (2022) 21:79. **https://doi.org/10.1186/s12943-022-01543-7**
2. Liquid biopsy in lung cancer: significance in diagnostics, prediction, and treatment monitoring. Li et al. Molecular Cancer (2022) 21:25. **https://doi.org/10.1186/s12943-022-01505-z**

Ordering Information

To place an order or to obtain additional information about our MagPrep® particles, buffers, and nucleic acid isolation kits, order online at **SigmaAldrich.com**.

For any interest in bulk or custom SKUs, contact your local Account Manager or place a request through our website.

Conditions of Sale

For research use and further manufacturing use only. Not for use in diagnostic procedures.

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