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Enabling Epigenetics Research

RapCap™ Beads - cfDNA Isolation, Plasma

Catalog No. 25505

(Version A1)

Contents

Overview

RapCap™ Beads are magnetic beads with a novel DNA-binding affinity chemistry for cfDNA isolation that are not silica- or ion exchange-based technology. By using this unique approach, cfDNA can be efficiently concentrated from trace amounts in various samples and volume sizes, providing a scalable soultion for low-abundance nucleic acid recovery. The RapCap™ Beads system also eliminates the use of harsh chemical treatments or precipiation steps, enabling size-based discrimination of nucleic acids, offering unique versatility compared to existing methods of cfDNA isolation. This streamlined approach not only reduces processing time and manual handling but also minimizes genomic DNA contamination, ultimately enhancing the sensitivity and reproducibility of downstream analyses like PCR and next-generation sequencing.

RapCap™ Beads Advantages:

- Simplified workflow
  - 40% reduction in total assay time, >65% reduction in hands-on-time
  - No ethanol, no drying - fully aqueous chemistry simplifies purification
  - No additional spin needed for plasma samples after thawing - genomic DNA (gDNA) levels already low
  - No protein carry-over (proteinase K digestion is not needed, no inhibitors present)
- Highly selective for cell free DNA (cfDNA)
  - 90 to 99% reduction in contaminating gDNA for plasma samples
  - 75% reduction in contaminating gDNA for saliva samples

Product	Format	Catalog No.
RapCap™ Beads - cfDNA Isolation, Plasma	50 rxns	25505

Kit Components and Storage

The kit contains sufficient reagents for 50 reactions. The reagents in this kit have multiple storage temperatures. Please store components according to the storage conditions below. All reagents are guaranteed stable for 6 months from date of receipt when stored properly.

Important: If crystallization of RapCap™ Binding Buffer is observed, place at 37°C for one hour with occasional agitation to redissolve crystals. Ensure all buffers are at room temperature before beginning.

Reagents	Quantity	Storage
RapCap™ Binding Buffer	100 mL	RT
RapCap™ Wash Buffer	50 mL	RT
RapCap™ Elution Buffer	1.25 mL	RT
RapCap™ Neutralization Buffer	125 µL	RT
RapCap™ Sample Conditioner	400 µL	RT
RapCap™ Beads	1.25 mL	4°C

Additional Materials Required

- Microcentrifuge
- End-over-end rotator
- Pipettes and tips (multi or single-channel P20, P200, and P1000)
- 200 µL PCR strip tubes
- Vortex
- Magnetic rack for 2 mL Eppendorf tubes.
- Magnetic tray for 200 µL strip tubes
- 2 mL Eppendorf or similar snap-cap tubes.

Optional items:

- TapeStation electrophoresis system (Agilent model 4200 or 4150)
- TapeStation Cell-free DNA Reagents (Agilent 5067-5631)
- TapeStation Cell-free DNA ScreenTape (Agilent 5067-5630)

Protocol

The following protocol is for Plasma.

Optional step: To reduce genomic DNA content in cfDNA isolations from plasma a pre-spin can be done to remove cellular fraction. It significantly shortens the protocol if customers using our kit can eliminate this step.

Centrifuge at 16,000 x *g* for 2 minutes at room temperature. Carefully remove liquid component without disturbing pelleted cellular material.

Repeat spin a second time to ensure removal of all cellular material.

Keep RapCap™ Beads at 4°C. During the protocol, if beads are out of the refrigerator for an extended period, place them on wet ice.

Note: Some bead clumping may be observed during plasma cfDNA binding or elution, but this will not affect performance.

Protocol

1. Place 1 mL plasma sample in nuclease free 2 mL Eppendorf tube.
2. Add 1 mL of RapCap™ Binding Buffer to each sample.
3. Vortex to fully resuspend RapCap™ Beads and add 25 µL to each sample.
4. Add 8 µL of RapCap™ Sample Conditioner for each 1 mL sample.
5. Vortex sample with beads, binding buffer and plasma for 20 seconds or until fully mixed.
6. Put sample tubes on end-over-end rotator for 30 minutes at room temperature.
7. Quick spin samples to remove liquid from lids. Place sample tube on magnetic rack and let sit at least 2 minutes to collect RapCap™ Beads to side. Carefully remove supernatant with clean 1 mL pipette tip avoiding removal of beads.
8. Add 1 mL of RapCap™ Binding Buffer to beads. Remove the tube from magnet and pipette up and down several times to resuspend beads.
9. Place the sample tube back on magnetic rack and let stand for 2 minutes until magnetic beads have collected, remove supernatant with clean 1 mL pipette tip.
10. Add 200 µL RapCap™ Wash Buffer to each sample. Remove tube from magnet and resuspend beads in Wash Buffer. Transfer RapCap™ Beads in RapCap™ Wash Buffer to a PCR strip tube.
11. Place strip tubes with RapCap™ Beads onto magnetic tray. When RapCap™ Beads are collected on the side, use multichannel pipette to remove RapCap™ Wash Buffer.
12. Add 200 µL Wash Buffer to each tube containing RapCap™ Beads. Lift strip from rack and resuspend RapCap™ Beads in Wash Buffer for second wash.
13. Place strip tubes with RapCap™ Beads onto magnetic tray. When RapCap™ Beads are collected by magnet use multichannel pipette to remove RapCap™ Wash Buffer.

14. Quick spin sample tubes to collect remaining liquid at bottom and place back on magnetic tray. Use P-20 pipette to remove residual liquid. Do not allow the beads to dry before continuing to elution step.
15. To each RapCap™ Beads sample add 22.5 µL (or 0.9 volumes final desired volume) of RapCap™ Elution Buffer and resuspend RapCap™ Beads and place on 55°C heat block for 5 minutes.
16. Label fresh PCR strip tubes and, for each sample, add 2.5 µL (or 0.1 volumes desired final volume) of RapCap™ Neutralization Buffer to each tube which will receive a sample.
17. Place samples on magnetic tray. Transfer supernatant without carrying over beads to fresh PCR strip containing RapCap™ Neutralization Buffer and pipette up and down several times to mix. Samples are now in 25 µL neutral 10 mM Tris and can be stored or used for downstream applications.

To quantify and QC cfDNA collected it is recommended to run 2 µL of the collected cfDNA sample on a TapeStation electrophoresis system using cfDNA screen tape following user instructions.

https://www.agilent.com/cs/library/usermanuals/public/cfDNA_QuickGuide.pdf

Technical Services

If you need assistance at any time, please call or send an e-mail to Active Motif Technical Service at one of the locations listed below.

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