MAGicBead™ cfDNA Isolation Kit

Catalog No. D4086
Require a Custom Solution? Inquire Here or email busdev@zymoresearch.com

ZYMO RESEARCH
The Beauty of Science is to Make Things Simple

QUICK PROTOCOL

Automation

- ✓ This product is compatible with all "open" platforms, including Kingfisher™ Flex/Apex (Thermo-Fisher Scientific), Fluent® X (Tecan), Microlab® STAR™ (Hamilton), OT-2 (Opentrons), and others.
- ✓ Email automation@zymoresearch.com for assistance, including scripts and other specific inquiries tailored to your project.

Sample and Buffer Preparation

- ✓ (Recommended) Remove any cryoprecipitates from thawed cell-free biofluid[1] samples by spinning at 12,000 *x g* for 10 minutes at room temperature (15-30 °C).
- ✓ Reconstitute lyophilized Proteinase K by adding 6.25 ml Storage Buffer to Proteinase K (125 mg), mix thoroughly by vortexing. Store reconstituted Proteinase K (20 mg/mL) in -20 °C.
- ✓ Store all other kit components at room temperature (15-30 °C).

Equipment Required

- ✓ An open automation platform (e.g., KingFisher™ Flex/Apex, Thermo-Fisher Scientific). For manual applications, a magnetic stand compatible with conical/centrifuge tubes/plates, a vortex, and a rotator capable of at least 30 rpm are needed.

cfDNA Extraction Procedure

Input Volume	MAGicBead™ cfDNA Digestion Buffer	Proteinase K	MAGicBead™ cfDNA Binding Buffer	MAGicBeads™ cfDNA	Final Reaction Volume
0.2 mL	50 µL	8 µL	50 µL	10 µL	**318 µL**
1 mL	250 µL	40 µL	250 µL	10 µL	**~1.6 mL**
2 mL	500 µL	80 µL	500 µL	10 µL	**~3.1 mL**
10 mL	2.5 mL	400 µL	2.5 mL	10 µL	**~15.5 mL**

1. Referring to the table (*above*), add a cell-free biofluid[1] sample into a clean tube that can comfortably accommodate (~70% capacity) the final reaction volume (*Note: for other sample volumes, scale other components proportionally EXCEPT the beads*[2]).
2. Add the **Digestion Buffer** and **Proteinase K**. Mix thoroughly by vortexing or pipetting for 5 seconds.
3. Digest lysate mixture according to the sample collection method (*below*):

Collection Tube	Digestion Condition
K_2EDTA, Na-Citrate, NaF/K-Oxalate, non-plasma biofluids[1]	37 °C for 30 minutes or RT for 2 hours
Streck Cell-Free DNA BCT®	55 °C for 30 minutes or RT for 2 hours
K_3EDTA and Na_2EDTA	37 °C for 60 minutes

4. Add the **Binding Buffer**. Mix thoroughly by vortexing or pipetting for 5 seconds.
   (The Binding Buffer must be added prior to MAGicBeads™ cfDNA)
5. Completely resuspend **MAGicBeads™ cfDNA** by vortexing and inverting vigorously until there is no clump of beads.
6. Add 10 µL **MAGicBeads™ cfDNA** and mix thoroughly by vortexing or pipetting for 5 seconds.
7. Incubate at room temperature with constant agitation[3] using a rotator (at ~30 rpm) for the sample input volumes indicated (*below*).

Sample Input Volume	Incubation Time
≤ 1 mL	5 minutes
> 1 mL and ≤ 3 mL	10 minutes
> 3 mL and ≤ 10 mL	20 minutes

8. After taking sample out of a rotator, flick sample tube down to move residual lysates to the bottom of the tube. Carefully open the cap prior applying them on magnetic stand to prevent loss of lysates.
9. Apply sample to a magnetic stand until beads are fully pelleted.
10. Carefully discard the supernatant, then remove sample from magnetic stand.
11. Add 800 µL the **Wash Buffer**, mix thoroughly well by pipetting[4].
12. Apply sample to a magnetic stand until beads are fully pelleted.
13. Carefully discard the supernatant, then remove sample from magnetic stand.
14. Repeat Steps 11 – 13 with 300 µL the **Wash Buffer** for two additional times.
15. Apply sample to a magnetic stand until beads are fully pelleted. Remove residual wash buffers by pipetting[5].
16. Add ≥ 15 µL the **Elution Buffer**[6] gently resuspend beads.
17. Incubate at room temperature for 1 minute.
18. Apply sample to a magnetic stand until beads are fully pelleted.
19. Carefully transfer the eluate to a clean microcentrifuge tube. The purified cfDNA is ready for immediate use or can be stored (-20 °C) for long-term storage.

Notes

1) Compatible with most cell-free biofluids, including plasma and serum derived from various blood collection tubes, urine, saliva, cerebrospinal fluid, amniotic fluids, spent cell culture media. **Proteinase K digestion efficiency** of plasma samples can vary depending on sample quality, types of anticoagulants used, and other preservatives used in collection. **Heparin tubes** are NOT compatible. However, eluates can be treated with Heparinase I (NEB, Cat# P0735S) or II (NEB, Cat# P0736S) and cleaned-up using DNA Clean & Concentrator (Zymo Research, Cat# D4013). **DNA/RNA Clean & Concentrators** are compatible. **Significantly hemolyzed plasma samples** may lead to pinkish eluates – simply reapply the protocol without proteinase K addition/incubation steps to clean-up. For **non-plasma sample types**, recommended digestion condition is: 55 °C for 15 minutes, then 37 °C for 15 minutes. **Viscous samples**, such as whole saliva and synovial fluid, may require spinning down to remove cellular debris or sample dilution with PBS to facilitate compatibility. Urine sample stored in **Urine Conditioning Buffer** (Zymo Research, D3061), can be pelleted; the pellet can then be resuspended in PBS (1 mL PBS per pellet, 40 mL urine per pellet) to serve as cell-free biofluid sample input.

2) For most cell-free biofluid samples, 10 µL **MAGicBeads™ cfDNA** will be sufficient to achieve high cfDNA yields. For samples expected to have yields > 100 ng, the bead input volume can be increased up to 30 µL. However, this will necessitate increasing the elution volume accordingly (1.5x the bead input volume; see below).

3) Beads can be eluted twice to further increase recovery. Apply a second round of the Elution Buffer (refer to Appendix B on Page 6 for recommended elution volume). Use fresh elution buffer for second elution instead of re-eluting with first eluate, then combine the two eluates.

Bead Input Volume	Elution Volume
10 µL	≥ 15 µL
20 µL	≥ 30 µL
30 µL	≥ 45 µL

3) Beads settle quickly and should be well mixed with sample lysate just prior to incubation. Instead of a rotator, vortex or rollers can be used at moderate speed that will keep beads resuspended during incubation.

4) Transferring beads resuspended in first wash buffer to a clean centrifuge tube can help make subsequent wash steps easier and achieve cleaner elution steps. 2ml centrifuge tubes is recommended.

5) Air-drying the beads prior to adding elution buffer is not necessary.

6) Minimum elution volume is bead input dependent. Please refer to Note #2 above for recommendations.

Ordering Information

Product Description	Catalog No.	Size
MAGicBead™ cfDNA Isolation Kit	D4086	2 mL input x 50 Prep.

- ✓ D4086 kit size is 50 preps with 2 mL sample input per prep.
- ✓ Require a Custom Solution? Inquire Here or email busdev@zymoresearch.com
- ✓ Email automation@zymoresearch.com for assistance, including scripts and other specific inquiries tailored to your project.