ZYMO RESEARCH

DNA Purification Made Simple™

MAGicBead™ cfDNA Isolation Kit

Novel DNA binding chemistry enabling unparalleled cfDNA extraction performance

Highlights

- Robust isolation of cfDNA from up to 10 mL plasma w/ unique MAGicBead™ technology
- High-quality cfDNA from plasma, saliva, urine, etc. is ideal for NGS
- Compatible with most automation platforms

Catalog Numbers:
D4086 (2 mL input x 50 Prep.)
Require a Custom Solution? Inquire Here
(or email busdev@zymoresearch.com)

Scan to view the online protocol/video.

Table of Contents

Product Contents

MAGicBead™ cfDNA Isolation Kit	Cat No. D4086 (2 mL input x 50 Prep.)	Storage Temperature
MAGicBead™ cfDNA Digestion Buffer	25 mL	Room Temp
MAGicBead™ cfDNA Binding Buffer	25 mL	Room Temp
Proteinase K & Storage Buffer	125 mg	Room Temp
MAGicBeads™ cfDNA	1.5 mL	Room Temp
MAGicBead™ cfDNA Wash Buffer	70 mL	Room Temp
MAGicBead™ cfDNA Elution Buffer	2 mL	Room Temp
Instruction Manual	1	

- ✓ D4086 kit size is 50 preps with 2 mL sample input per prep.
- ✓ Email automation@zymoresearch.com for assistance on D4086, including scripts and other specific inquiries tailored to your project.

Specifications

- **Purity –** Eluted cfDNA is NGS-ready. NOTE: Due to low levels of cfDNA available in cell-free biofluids, a spectrophotometer (e.g., NanoDrop) is not recommended for quantification or purity assessment[1].

- **Yield –** Typical yields range from 0.5 ng to 20 ng from healthy donor plasma samples[2]. This can range from 0.1 ng to 100 ng depending on the health status of donors.

- **Fragment Sizes –** For plasma samples[2], DNA fragments will typically display a mono-nucleosomal peak in the 140-170 bp range and a genomic DNA fragments peak > 10 kb. However, other multi-nucleosomal apoptotic DNA fragments may also be present.

- **Elution Volume –** ≥ 15 µl MAGicBead™ cfDNA Elution Buffer.

- **Processing Time –** 1-1.5 hour (~15 minutes hands-on time).

- **Sample compatibility –** Plasma samples derived from most blood collection tube types are compatible, including Streck Cell-Free DNA BCT®. Plasma samples derived from blood collection tubes with heparin are NOT compatible. Highly viscous biofluids, such as whole saliva and synovial fluids, may have limited compatibility. Refer to Appendix A (Page 6) for more details.

  Required Equipment – An open automation platform: KingFisher Flex/Apex (Thermo-Fisher Scientific), Fluent® X (Tecan), Microlab® STAR™ (Hamilton), OT-2 (Opentrons) and others. For manual applications, a magnetic stand[3] compatible with conical/centrifuge tubes/plates, a vortex, and a rotator capable of at least 30 rpm are needed.

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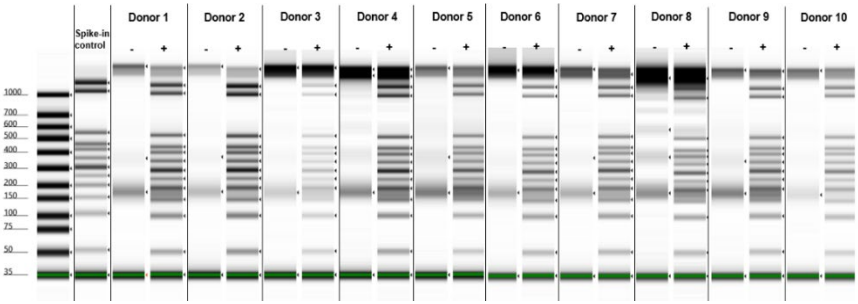
1. Spectrophotometers (e.g., NanoDrop) requires a minimum of 10 ng/µl for reliable quantification and purity assessment, which cell-free biofluids rarely yield. However, cfDNA isolated using this kit is compatible with downstream assays, including NGS, bisulfite sequencing, and qPCR.
2. cfDNA yield ranges and fragment profiles for cell-free biofluid types other than plasma (e.g., urine, saliva, etc.) have not been well established.
3. Recommended magnetic stands: Promega MagneSphere® Magnetic Separation Stand or Permagen® Centrifuge Magnetic Separation Rack

Product Description

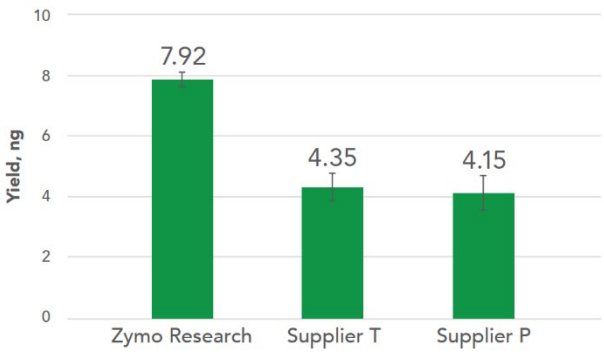
The **MAGicBead™ cfDNA Isolation Kit** features a unique, magnetic bead surface chemistry that facilitates unparalleled cfDNA recovery from biofluids. Input volumes can range up to 10 mL of plasma. It is compatible with all open automation platforms and a wide range of sample types (serum, saliva, urine, etc.). This kit features a simple, streamlined extraction process with minimal number of steps and does not require the bead drying step common with other similar procedures. Eluted cfDNA is free of enzymatic inhibitors that can interfere with PCR or other sensitive downstream applications, including Next-Gen Sequencing.

Qualitative Fragment Size Analysis



Cell-free DNA was extracted from 10 different healthy donor plasma samples (1mL) using **MAGicBead™ cfDNA Isolation Kit** (Zymo Research). Performed in duplicates, one of each was spiked with 30ng of 50bp DNA Ladder (Zymo Research, M5001) for yield recovery assessment and visualized using Cell-free DNA ScreenTape Analysis (Agilent Technologies)

Highest cfDNA Yield



Cell-free DNA was extracted (from 1mL Plasma) using three different magnetic-bead based extraction kits: **MAGicBead™ cfDNA Isolation Kit** (Zymo Research) and a kit from supplier T and supplier P. Total cfDNA yield assessment using Qubit™ 1x dsDNA HS Assay Kit (Thermo-Fisher Scientific).

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 vortex. Store reconstituted Proteinase K (20mg/ml) in 20 °C.
- ✓ Store all other kit components at room temperature (15-30 °C).

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 and 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

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. Please refer to Appendix A (Page 6) for details on compatibility of various sample types.
2. Bead input is dependent on expected total yield; please refer to Appendix B (Page 6) for recommendations.

cfDNA Extraction Procedure (Cont.)

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[1].
7. Incubate at room temperature with constant agitation[2] using a rotator (recommended ~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 ≤ 10mL	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 to 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 by pipetting[3].
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 buffer by pipetting[4].
16. Add ≥ 15 µL the **Elution Buffer**[5] and 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.

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1. Bead input is dependent on expected total yield; please refer to Appendix B (Page 6) for recommendations.
2. 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.
3. Transferring beads resuspended in first wash buffer to a clean centrifuge tube can help make subsequent wash steps easier and achieve cleaner elution steps. 2 ml centrifuge tubes is recommended.
4. Air-drying the beads prior to adding elution buffer is not necessary.
5. Minimum elution volume is bead input dependent. Please refer to Appendix B (Page 6) for recommendations.

Appendices

Appendix A

Sample Compatibility

Proteinase K digestion efficiency of plasma samples can vary depending on sample quality, types of anticoagulants used, and other preservatives used in collection. Typical collection methods listed on Page 4 include specific incubation time and temperature for optimizing the digestion step.

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; refer to Troubleshooting (Page 8) for more detail.

For **non-plasma sample types** (serum, saliva, urine, cerebrospinal fluids, amniotic fluids, spent cell culture media, pleural fluid, bile acid, bronchoalveolar lavage, etc.), recommended digestion condition is: 55 °C for 15 minutes, then 37 °C for 15 minutes. Adjustments to this temperature/time, specific to the sample type used, can then be evaluated after this condition has been tested.

Viscous samples, such as whole saliva and synovial fluid, may have limited compatibility with this kit. Spinning down cellular debris or sample dilution with PBS may facilitate compatibility. Urine samples 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.

Appendix B

Bead Inputs and Elution Volumes

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 (minimum 1.5x the bead input volume; see below).

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

Troubleshooting

Observation	Possible Causes and Suggested Solutions
Low Yield	Cell-free biofluids are typically very low-yield in nature. For example, 1 mL plasma sample from a typical healthy donor will yield approximately 0.5-20 ng. Incomplete sample digestion will lead to low yield. Ensure that lysate is visibly transparent after proteinase K digestion. If lysate looks turbid, extend digestion time up to 30 minutes at 37 °C; more time or higher temperature is not recommended. MAGicBeads™ cfDNA must be added last prior to binding step. If the beads are added to the binding buffer alone, they will become inactive. Use fresh beads and buffer if this occurs. Beads can be eluted twice to further increase recovery. Apply a second round of **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. Ensure appropriate quantification methods are used. Qubit™ 1x dsDNA High Sensitivity Assay (Thermo-Fisher Scientific) is recommended method of quantification. Cell-free DNA ScreenTape Analysis (Agilent) is recommended for fragments size assessment. A spectrophotometer (e.g., NanoDrop) is NOT recommended for yield and purity assessment.

Observation	Possible Causes and Suggested Solutions
Beads clumping in sample mixture	Incomplete sample digestion will lead to proteins and cell debris to bind and clump beads. Digest samples in appropriate condition indicated in the **Protocol** section on Page 4. Ensure that lysate is visibly transparent after proteinase K digestion. If lysate looks turbid, extend digestion time up to 30 minutes at 37 °C.
Pink/Yellow Eluates	If the input plasma sample is significantly hemolyzed, eluates may look pink or yellow. Simply clean-up by resuspending the eluate in 1 mL DNase/RNase-Free Water and reapply the protocol without addition of Proteinase K and digestion step (no delay between addition of the Digestion Buffer and the Binding Buffer).

Feedback

How did this kit perform?

Did MAGicBead™ cfDNA fulfill
expectations required for your cell free research?

Let us know by filling out the feedback form here.
Or scan the QR code:

MAGicBead™ cfDNA Feedback Form

Ordering Information

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

- ✓ 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.

Individual Kit Components	Catalog No.	Amount
MAGicBead™ cfDNA Digestion Buffer	D4086-1-25	25 ml
MAGicBead™ cfDNA Binding Buffer	D4086-2-25	25 mL
Proteinase K Set, 125mg	D3001-2-125	125 mg
MAGicBeads™ cfDNA	D4086-3-1.5	1.5 mL
MAGicBead™ cfDNA Wash Buffer	D4086-4-70	70 mL
MAGicBead™ cfDNA Elution Buffer	D4086-5-2	2 mL

Notes

Notes

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