cfDNA Purification Kit (Magnetic Beads)

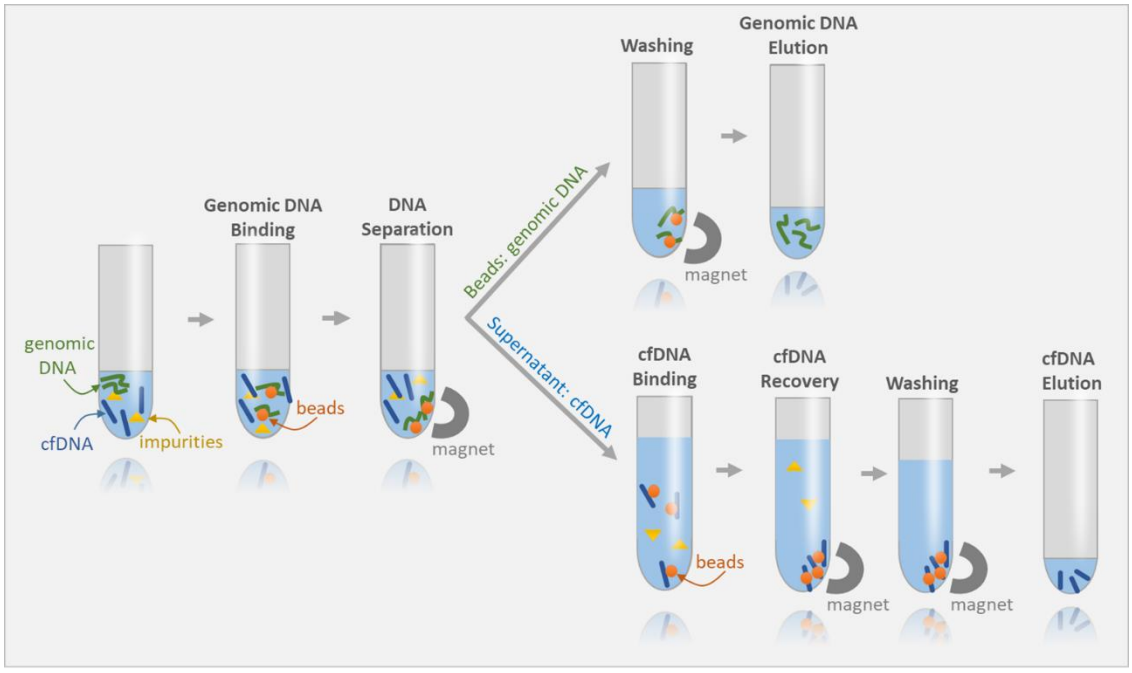
Catalog No.	40056S	40056L
Runs*	24	96

*Based on 50 µl of sample volume

Description

The **cfDNA Purification Kit (Magnetic Beads**) was developed for cell free DNA (cfDNA) enrichment by separating genomic DNA and blood DNA-derived fragments from cfDNA. Silica-based magnetic bead cfDNA isolation kits typically yield a large variation in cfDNA that includes the presence of genomic DNA that often depends on tumor stage, tumor size, or healthy status individuals. Most of the commercial cfDNA isolation kits can't specifically recover the cfDNA while leaving the high molecular weight genomic DNA behind. The presence of genomic DNA can lead to decreased sensitivity or inconsistent results in downstream applications such as next-generation sequencing (NGS), PCR, QPCR, and digital PCR etc. Therefore, an additional purification step to enrich cfDNA before downstream methods helps to improve signal from fragments that originate from cancer cells.

This kit uses Dual Solid Phase Reversible Immobilization (SPRI) technology for cfDNA purification. Most Dual SPRI procedures do NOT recover fragments below 100 bp. The kit separates cfDNA (50-500 bp) and genomic DNA, and recovers of 90% of the cfDNA without the high molecular weight genomic DNA with high efficiency. Fragments at 500 bp and above may also be retained. Both the 50-500 bp and >500 bp DNA fractions can be used for downstream applications such as single-stranded or double stranded NGS library prep, qPCR, ddPCR, and other methods.



Features

- Separation of cfDNA and genomic DNA; Recovery of both types of DNA
- Recovery of cfDNA (50-500 bp)
  - As short as 50 bp can be recovered
- Recovery of high molecular weight genomic DNA
- Removal of unwanted components and other impurities

Component

Catalog No.	40056S	40056L
CP1 Beads	0.6 mL	2.4 mL
CP2 Beads	2.1 mL	8.4 mL
EM Buffer	2.1 mL	8.4 mL

Storage Condition

- Store at 4°C, stable up to 12 months.

Reagent & Equipment Needed (not provided in this reagent)

- Magnetic particle concentrator
- 96-well plates or 96-deep well plates, microfuge tubes
- 80% ethanol (prepare before use)

Protocol

Note: Invert or shake the beads bottles thoroughly to resuspend the beads.

1) Transfer samples eluate from a cfDNA extraction kit to a 96-well plate. **Note**: The popular automated extraction instruments often vary in eluate volume and may need to be adjusted so equal volume of beads may be added in Step 2.
2) Add a 0.5X volume of the CP1 Beads to the wells or tubes containing samples (see Beads volume calculation table below). Slow pipetting of the viscous beads is needed for precise aliquot. Mix by pipetting gently and thoroughly with a multichannel pipettor. As an alternative, cap tubes or use a plate seal and vortex to thoroughly mix beads. Incubate for 5 min. **Note**: Briefly spin down plate or tubes if beads are stick to the wall of the wells or tubes.
3) Load the sample on a magnet, incubate for 3 min, and transfer the supernatant to a new 96-well plate, 96-deep well plate, or microfuge tubes dependent on the maximal solution volume needed (see Beads volume calculation table below).
4) **Optional**: If purification of genomic DNA is needed, add 200-500 µl of 80% ethanol to the old 96-well plate (containing beads) without disturbing the beads.

Note: The new 96-well plate with supernatant contains cfDNA. The old 96-well plate with the beads contains high molecular weight genomic DNA and DNA >500 bp. If purification of both genomic DNA and cfDNA is needed, we recommend starting the cfDNA recovery step before working with the genomic DNA plate.

For cfDNA recovery: the new 96-well plate with supernatant

5) Make master mix based on the volume of the samples: Add 1.7X volume of CP2 Beads and 1.7X volume of EM Buffer to a tube and vortex briefly (see Beads volume calculation table below). Slow pipetting of the viscous beads is needed for precise aliquot. Total 3.4X volume of the mix will be made. Process to the next step immediately after the master mix is made.
6) Add 3X volumes of the above master mix to the wells or tubes containing supernatants. Mix by pipetting gently and thoroughly. Capping or sealing the deep well plate and vortexing may be used as an alternative way to mix beads. Incubate for 20 min. (**Optional**: while incubation proceeds, genomic DNA recovery may be completed at steps 12-15).
7) Briefly spin down plate or tubes if beads are stick to the wall of the wells or tubes. Load the sample plate on a magnet, incubate for 5 min, and discard the supernatant carefully.
8) Add 200-500 µl of 80% ethanol without disturbing the beads. Incubate for 1 min and discard the supernatant carefully. Remove all residual ethanol without disturbing the beads.
9) Air-dry the beads on the magnet for 5 min.

10) Remove the plate from the magnet and resuspend the beads in at least 20 µl of water, 10 mM Tris-HCl, or low TE buffer to elute cfDNA from the beads. **Note**: Resuspension of the beads in less than 20 µl will reduce the yield. Tubes may be capped or plate sealed to resuspend beads by vortexing. A brief centrifugation step may improve bringing eluates to the bottom of the tube or wells before placing on the magnet.
11) Load the plate on the magnet, incubate for 1 min, and transfer supernatant (containing sample) to a new plate or tubes without disturbing the beads.

For genomic DNA recovery (optional): the old 96-well plate with beads

12) Discard the supernatant carefully. Remove all residual ethanol without disturbing the beads.
13) Air-dry the beads on the magnet for 2 min.
14) Remove the plate from the magnet and resuspend the beads in at least 20 µl of water, Tris-HCl (10 mM), or low TE buffer. **Note**: Resuspend the beads in less than 20 µl will reduce the yield.
15) Load the plate on the magnet, incubate for 1 min, and transfer supernatant (containing sample) to a new tube without disturbing the beads.

Beads volume calculation table

Sample volume	CP1 beads volume (0.5X of sample)	CP2 Beads volume (1.7X of sample)	EM Buffer volume (1.7X of sample)	Final volume (sample+CP1+CP2+EM)
40 µl	20 µl	68 µl	68 µl	180 µl
50 µl	25 µl	85 µl	85 µl	225 µl
60 µl	30 µl	102 µl	102 µl	270 µl
80 µl	40 µl	136 µl	136 µl	360 µl

cfDNA concentration and sizing

Determine cfDNA concentration by BioDynami dsDNA Quantification High Sensitivity Kits (Cat. # 40042 for Qubit fluorometer; Cat. # 40540H for microplate reader). Sizing of both cfDNA and genomic DNA fractions can be determined on Agilent Tapestation cfDNA tape kits. If genomic DNA is not required to be measured, cfDNA size can also be determined on HS DNA 1000 tape kits. cfDNA is now ready for downstream applications.

Quality Control

Magnetic beads components passed stringent functional quality test.

Product Use Limitation

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