Cell-Free DNA (cfDNA) Purification Kit

(version A4)

Catalog No. 25503
Catalog No. 25504

Revision	Date	Description of Change
A4	December, 2024	The elution volume for 10 ml of plasma has been updated to 100 ul in Section E. Elution.

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Overview

Cell-free DNA can serve as a valuable source of information for non-invasive detection and monitoring of various disease states. However, cell-free DNA is often found in limited amounts in plasma and serum samples. Active Motif's Cell-Free DNA (cfDNA) Purification Kit provides a quick, simple method to isolate and concentrate lower molecular weight cell-free DNA from larger nucleic acids within the sample.

The Cell-Free DNA (cfDNA) Purification Kit is designed to isolate cell-free DNA from human plasma/serum samples. The fast, magnetic bead-based protocol isolates cell-free DNA from fresh or frozen plasma that is suitable for use in downstream applications such as qPCR, ddPCR, DNA methylation analysis, and Next generation sequencing (NGS).

The Cell-Free DNA (cfDNA) Purification Kit provides enough reagents to isolate cell-free DNA from up to 100 ml or 250 ml human plasma. Easily process input volumes from 100 µl to 10 ml, or combine with automation to streamline handling of multiple samples.

product	format	catalog no.
Cell-Free DNA (cfDNA) Purification Kit	100 ml 250 ml	25503 25504

Kit Performance and Benefits

The Cell-Free DNA (cfDNA) Purification Kit can be used to isolate cell-free DNA from human plasma samples. There are two package sizes for this kit, which enable isolation from up to 100 ml and 250 ml human plasma respectively.

Input amounts: Both fresh and frozen plasma can be used with the Cell-Free DNA Purification Kit in the range of 100 µl to 10 ml. However, fresh plasma tends to have higher yields.

Plasma from blood collected with Streck Cell-Free DNA BCT® Tubes must go through a Proteinase K treatment prior to cell-free DNA isolation to ensure optimal yields. Omitting the Proteinase K treatment may decrease yields up to 50%.

Expected yield: The yield of cell-free DNA is approximately 1 - 100 ng per ml of plasma. Quantification by absorbance measurement (*e.g.* Nanodrop) may not be sensitive enough to accurately determine yield. We recommend using the Qubit™ dsDNA High Sensitivity Assay to quantify purified cfDNA.

Assay time: As little as 1.5 hours.

Cell-Free DNA (cfDNA) Purification Kit

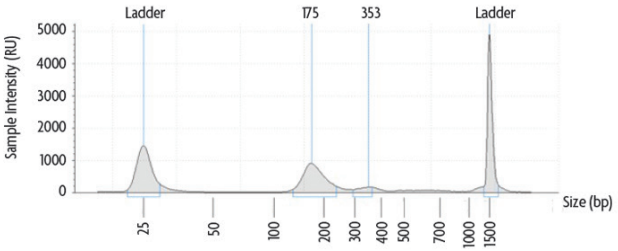

Figure 1: TapeStation determination of isolated cfDNA size.

The Cell-Free DNA (cfDNA) Purification Kit was used to isolate cell-free DNA from 5 ml of healthy human plasma. Isolated DNA was run on a TapeStation to assess the size of the purified DNA. Results show the majority of the purified samples is cell-free DNA at the expected size of 150-200 bp.

Plasma ID	Total DNA Recovered (ng)
A	7.00
A	7.05
B	32.45
B	33.50
C	26.80
C	27.70
D	10.90
D	10.90

Figure 2: Reproducibility of the Cell-Free DNA (cfDNA) Purification Kit.

The Cell-Free DNA (cfDNA) Purification Kit was used to isolate DNA from 1 ml of plasma from four different human donors in duplicate. The table above shows the total yield recovered from each sample with strong correlation between duplicates.

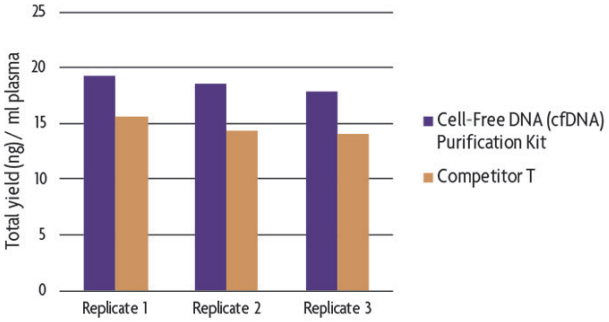

Figure 3: Automation compatibility with KingFisher™ Flex Purification System.

Both the Cell-Free DNA (cfDNA) Purification Kit and a competitor magnetic bead-based cfDNA purification kit (Competitor T) were used to isolate DNA from 600 µl of plasma from a normal human donor in triplicate using the KingFisher Flex Purification System. Results show the total DNA yield (ng) per ml of plasma. The Cell-Free DNA (cfDNA) Purification Kit recovered more DNA than the alternative purification method.

Kit Components and Storage

Cell-Free DNA Purification Kits are for research use only. Not for use in diagnostic procedures. All components are guaranteed stable for 6 months from date of receipt when stored properly.

Reagents	Quantity 100 ml kit	Quantity 250 ml kit	Storage
Lysis/Binding Buffer AM2	115 ml	3 x 95 ml	RT
Wash Buffer AM8	2 x 55 ml	5 x 55 ml	RT
Elution Buffer AM6	6 ml	15 ml	RT
Magnetic Bead Solution	2 x 1.33 ml	5 x 1.33 ml	RT

Additional materials required but not supplied

- Human plasma
- Proteinase K (20 mg/ml) if plasma samples were from blood collected using Streck Cell-Free DNA BCT® tubes
- 20% SDS solution if plasma samples were from blood collected using Streck Cell-Free DNA BCT® tubes
- Pipettes and tips
- Vortex
- Magnetic stand compatible for use with magnetic particles in microcentrifuge tubes. Depending on plasma volume, magnetic stand compatible with 15 ml or 50 ml conical tubes may also be used.
- 1.5 ml low retention microcentrifuge tubes (*e.g.* Eppendorf™ LoBind Microcentrifuge tubes)
- Microcentrifuge
- Fresh 100% ethanol

Buffer Preparation and Recommendations

Lysis/Binding Buffer AM2

The Lysis/Binding Buffer AM2 is shipped as a concentrate and requires the addition of 100% ethanol (EtOH) prior to use. Once ethanol is added, place a mark on the bottle to indicate it is a complete solution and mix by inverting gently. If a precipitate is present, incubate the solution at 37°C for 30 minutes. Close bottle tightly for long term storage and store at 4°C for up to one year.

	100 ml Kit (per bottle)	250 ml Kit (per bottle)
Lysis/Binding concentrate	115 ml	95 ml
100% EtOH	23 ml	19 ml

Wash Buffer AM8

Wash Buffer AM8 is shipped as a concentrate and requires the addition of 100% ethanol (EtOH) prior to use. Once ethanol is added, place a mark on the bottle to indicate it is a complete solution and mix by inverting gently. If a precipitate is present, incubate the solution at 37°C for 30 minutes. Close bottle tightly for long term storage and store at 4°C for up to one year.

	100 ml Kit (per bottle)	250 ml Kit (per bottle)
Wash Buffer concentrate	55 ml	55 ml
100% EtOH	51 ml	51 ml

80% EtOH

Prepare a fresh 80% ethanol (EtOH) solution prior to each extraction. Each sample will need a minimum of 2 ml 80% ethanol solution for washing steps.

Magnetic Bead Solution

Is provided ready to use. Mix beads prior to use. There should be no visible sedimentation at the bottom of the solution after mixing. Beads will settle quickly, so be sure to mix the magnetic bead solution after adding to each sample. Failure to do so may result in inconsistent results.

Elution Buffer AM6

Is provided ready to use.

Assay Protocol

Read the entire protocol before use.

Prepare the reagents needed for the assay according to the instructions provided in the Buffer Preparation and Recommendations protocol. Follow the instructions below to determine the volume of reagents needed based on the amount of plasma used per sample.

Section A: Experimental Set-Up

The Cell-Free DNA (cfDNA) Purification Kit provides a protocol based on the amount of plasma to be used for the purification. Any amount from 100 µl to 10 ml of fresh or frozen human plasma can be used. Scale buffer and magnetic bead solution volumes accordingly using the table below.

Plasma volume	Lysis/Binding Buffer AM2	Magnetic Bead Solution	Tube size
x (x = ml of plasma)	1.25x	0.025x	N/A
0.5 ml	625 µl	12.5 µl	2 ml
1 ml	1.25 ml	25 µl	15 ml
5 ml	6.25 ml	125 µl	15 ml or 50 ml*
10 ml	12.5 ml	75 µl**	50 ml

*Using a 50 ml tube for 5 ml or more of plasma is recommended over a 15 ml tube. While a 15 ml tube will work it may lead to slightly lower yields.

**When working with 10 ml of plasma per tube, the recommended amount of Magnetic Bead Solution differs from the recommended amounts per ml of plasma used. Use the volume provided in the table for cfDNA purification from 10 ml plasma.

(Optional) Proteinase K Treatment:

Proteinase K Treatment is required if samples were collected using Streck Cell-Free DNA BCT® tubes. Omission of Proteinase K treatment may decrease yields by 50%. If blood was not collected using Streck Cell-Free DNA BCT® Tubes, proceed to Section B: Lysis/Binding.

Plasma volume	Proteinase K	20% SDS Solution
x (x = ml of plasma)	0.015x	0.05x
0.5 ml	7.5 µl	25 µl
1 ml	15 µl	50 µl
5 ml	75 µl	250 µl
10 ml	150 µl	500 µl

1. Add the appropriate amount of plasma to an appropriately sized tube(s).
2. Add 15 µl Proteinase K (20 mg/ml) for every 1 ml of plasma used.

3. Add 50 µl 20% SDS solution for every 1 ml of plasma used.
4. Mix by inverting gently 5 times.
5. Incubate at 60°C for 20 minutes.
6. After incubation, place tube(s) on ice for 5 minutes to cool to room temperature.
7. Once tube(s) have reached room temperature proceed directly into Section B: Lysis/Binding.

Section B: Lysis/Binding

1. Add the appropriate amount of plasma to the recommended sized tube(s).
2. Add 1.25 ml of complete **Lysis/Binding Buffer AM2** (with EtOH added) for every 1 ml plasma.
3. Add 25 µl **Magnetic Bead Solution** for every 1 ml of plasma used.

   Note: Mix bead solution prior to added to tubes. There should be no visible sedimentation at the bottom of the solution after mixing. Beads will settle quickly so it is important to mix the magnetic bead solution after adding to each sample. Failure to do so may result in inconsistent yields.

4. Vortex or shake tube(s) vigorously for 10 minutes at room temperature.

   Note: To obtain high yields, ensure that the plasma/buffer solution is mixing vigorously. A vortexing mixer with a tube-holder is recommended.

5. Place tube(s) in a magnetic stand for 5 minutes, or until the solution clears.
6. While keeping the tube(s) on the magnetic stand, remove and discard the supernatant. Be careful not to disturb the magnetic particles.
7. Keep tube(s) on the magnetic stand an additional minute. Then, remove and discard any residual supernatant.

Section C: First Wash

1. Remove the tube(s) from the magnetic stand and add 1 ml of complete **Wash Buffer AM8** (with EtOH added) to each Lysis/Binding tube.

   Note: The same volume of buffer is used regardless of the tube size.

2. For 2 ml and 15 ml tubes, resuspend the magnetic beads by vortexing for 10 seconds or by pipetting up and down 6 times. For 50 ml tubes, resuspend beads by swirling tube or pipetting up and down 10 times.
3. Transfer each magnetic bead suspension into a low retention 1.5 ml microcentrifuge tube.
4. Place tube(s) in a magnetic stand for 10-30 seconds to allow the beads to aggregate to the side of the tube(s).
5. Collect supernatant in pipette and use it to wash the original Lysis/Binding sample tube to collect any remaining material. Transfer this solution back to the 1.5 ml microcentrifuge on the magnetic stand and let sit for 10-30 seconds, or until solution is clear.

6. Using a P1000 pipette, remove and discard as much supernatant as possible.
7. Tap magnetic stand on bench 5 times to dislodge any remaining liquid. Using a P200 pipette, remove any remaining buffer.
8. Remove tube(s) from the magnetic stand. Add 1 ml of complete **Wash Buffer AM8** (with EtOH added) to each tube.
9. Resuspend the magnetic beads by vortexing for 20 seconds or pipetting up and down 6 times.
10. Quick spin the tubes in a microcentrifuge to collect the liquid to from the cap.
11. Place tube(s) on a magnetic stand for 10-30 seconds.
12. Using a P1000 pipette, remove and discard as much supernatant as possible.
13. Tap magnetic stand on bench 5 times to dislodge any remaining liquid. Using a P200 pipette, remove any remaining buffer.

Section D: Second Wash

1. Remove the tube(s) from the magnetic stand and add 1 ml fresh **80% EtOH**.
2. Resuspend the magnetic beads by vortexing for 20 seconds or by pipetting up and down 6 times.
3. Quick spin the tubes in a microcentrifuge to collect the liquid to from the cap.
4. Place tube(s) in a magnetic stand for 10-30 seconds, or until solution is clear.
5. Using a P1000 pipette, remove and discard as much supernatant as possible.
6. Tap magnetic stand on bench 5 times to dislodge any remaining liquid. Using a P200 pipette, remove any remaining ethanol.
7. Remove tube(s) from the magnetic stand. Add 1 ml fresh **80% EtOH** to each tube.
8. Resuspend the magnetic beads by vortexing for 20 seconds or piepting up and down 6 times.
9. Quick spin the tubes in a microcentrifuge to collect the liquid to from the cap.
10. Place tube(s) on a magnetic stand for 10-30 seconds.
11. Using a P1000 pipette, remove and discard as much supernatant as possible.
12. Tap magnetic stand on bench 5 times to dislodge any remaining liquid. Using a P200 pipette, remove any remaining ethanol.
13. Leave the tubes on the magnetic stand with the cap(s) open for 2 minutes. Then, tap magnetic stand on bench 5 times to dislodge any remaining liquid. Using a P20 pipette, remove any remaining EtOH.
14. Allow magnetic particles to air dry for an additional 1-3 minutes.

    Note: Be careful not to over-dry. Over-drying may cause the beads to stick to the tube, leading to a lower yield.

Section E: Elution

1. Remove the tube(s) from the magnetic stand. and add the desired volume of **Elution Buffer AM6** to each tube. A minimum of 12.5 µl Elution Buffer AM6 per ml of plasma is recommended to elute DNA to ensure optimal yields.

Plasma volume	Elution Buffer AM6
x (x = ml of plasma)	0.0125x
0.5 ml	6.25 µl
1 ml	12.5 µl
5 ml	62.5 µl
10 ml	100 µl**

**When working with 10 ml of plasma per tube, the recommended amount of Elution Buffer AM6 differs from the recommended amounts per ml of plasma used. Use the volume provided in the table for cfDNA purification from 10 ml plasma.

2. Vortex tube(s) vigorously for 5 minutes.
3. Quick spin the tubes in a microcentrifuge to collect the liquid to from the cap.
4. Place tube(s) on a magnetic stand for 10-30 seconds.
5. Transfer eluted cell-free DNA into a new microcentrifuge tube.
6. Store DNA at -20°C, or proceed directly with downstream analysis.

Note: Plasma will yield 1 - 100 ng of cell-free DNA per ml of plasma. Quantification by absorbance measurement (**e.g.** Nanodrop) may not be sensitive enough to accurately determine yields. Instead, we suggest using Qubit™ dsDNA High Sensitivity Assay.

Cell-free DNA is small in size (~170 bp). Therefore, PCR primers should be designed to produce amplicons of 150 bp or less. A higher number of amplification cycles (40 cycles) may be needed in some cases.

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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