User's Manual and Instructions

cfPure™ Cell-Free DNA Extraction Kit

Catalog Number: K5011610, K5011625

Storage Conditions
Store all of the contents of this kit at room temperature

Shelf Life
1 year from the date of receipt under proper storage conditions

Features
- Non-toxic chemicals
- High Cell-Free DNA recovery
- Short and Scalable Protocol
- Purified DNA is suitable for NGS, PCR, Bisulfite sequencing, etc

Description
The cfPure™ Cell-Free DNA extraction kit allows for fast and efficient Cell-Free DNA (cfDNA) isolation from plasma/serum samples. The magnetic bead-based extraction protocol is ideally suited for use with robotic liquid handlers. DNA extracted using this kit is suitable for PCR, qPCR, next generation sequencing (NGS), and other applications.

Contents
All necessary reagents for cfDNA isolation from human plasma samples are provided. There are 2 package sizes for this kit, which contains sufficient reagents for isolating cfDNA from up to 100 ml and 250 ml of human plasma, respectively.

Quality Control
Each component has been tested for purity and efficacy.

Important Notes
Starting Material: Both fresh and frozen plasma can be used with the Cell-Free DNA isolation protocol. Fresh plasma, however, tends to have higher yields.

Quantification: Plasma will yield 1-100 ng of Cell-Free DNA per ml of plasma. Therefore, quantification by absorbance measurement (eg. Nanodrop) may not be sensitive enough to accurately determine yield. Instead, we suggest using Qubit™ dsDNA High Sensitivity Assay.

Recommendations for PCR: Due to the highly fragmented nature of the nucleic acids obtained from plasma, care should be taken in the design of primers. Cell-free DNA tends to have a small size (~170bp). Therefore, PCR primers should be designed to produce amplicons of 150 bp or less. Given the low concentration of cfDNA in plasma taken from healthy individuals, 40 amplification cycles may be needed in some cases.

Streck Cell-Free DNA BCT Tube(s): Plasma from blood collected with Streck Cell-Free DNA BCT Tube(s) must go through a Proteinase K treatment prior to Cell-Free DNA isolation to ensure optimal yields. Forgoing Proteinase K treatment may decrease yields by 50%

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Equipment and Reagents to be Supplied by User

- Pipettes
- Vortex-Genie 2 or similar vortexing mixer*
- Magnet stand for molecular applications (e.g. DynaMag™-15 or DynaMag™-2)
- 1.5 ml non-stick eppendorf tube(s)
- Fresh 100% EtOH

* Contact AMSBIO Technical Service for additional recommendations for high throughput or automated mixing.

Protocol

Prior to Initial Use

The Lysis/Binding and Wash Buffer are shipped as a concentrate. If precipitate is present in either solution, incubate solution at 37°C for 30 minutes. 100% EtOH must be added to both solutions before the first use. Once EtOH is added, these buffers are stable for one year if stored at 4°C. Be sure to close the bottle tightly for long term storage.

For 100 ml kit (K5011610)

- Add 23 ml of fresh 100% ethanol to each bottle of Lysis/Binding Buffer and mix by inverting gently
- Add 51 ml of fresh 100% ethanol to each bottle of Wash Buffer and mix by inverting gently
- Prepare fresh 80% ethanol solution prior to each extraction

For 250 ml kit (K5011625)

- Add 19 ml of fresh 100% ethanol to each bottle of Lysis/Binding Buffer and mix by inverting gently
- Add 51 ml of fresh 100% ethanol to each bottle of Wash Buffer and mix by inverting gently
- Prepare fresh 80% ethanol solution prior to each extraction

Before starting the protocol, determine the amount of plasma to be used for extraction and calculate the amount of buffer and beads needed. Any amount from 100 µl to 10 ml of plasma can be used. Scale buffer and bead volumes accordingly using the table below.

Plasma	Lysis/Binding Buffer	Bead Solution	Tube(s) size
x (x=ml of plasma)	1.25x	0.025x	n/a
500 ul	750 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 50ml*
10 ml	12.50 ml	250 µl	50 ml

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

Proteinase K Treatment

If samples were collected using a **Streck Cell-Free DNA BCT tube(s)**, Proteinase K treatment is required to ensure optimal yields. If blood was not collected with **Streck Cell-Free DNA BCT tube(s)** proceed to the Lysis/Binding step.

Plasma	Proteinase K	20% SDS Solution
x (x=ml of plasma)	0.015 x	0.050 x
500 µl	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 of Proteinase K (20 mg/ml) for every 1 ml of plasma used
3. Add 50 µl of 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 tube(s) to room temperature
7. Once tube(s) are at room temperature proceed to step 2 of the Lysis/Binding section

Lysis/ Binding

1. Add the appropriate amount of plasma to appropriately sized tube(s)
2. Add 1.25 ml of **cfPure Lysis/Binding Buffer** for every 1 ml of plasma used
3. Add 25 µl of **cfPure Magnetic Bead Solution** for every 1 ml of plasma

   Important: Mix beads well prior to adding. 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 yields
4. Vortex or shake tube(s) vigorously for 10 minutes at room temperature

   * To obtain high yields, ensure that plasma/buffer solution is mixing vigorously in tube(s). A vortexing mixer with a tube(s)-holder that allows for walk-away mixing will make this easier.
5. Place tube(s) into a magnet stand for 2 to 5 minutes, or until solution clears
6. While keeping the tube(s) on the magnet stand, remove supernatant. Be careful not to remove magnetic particles
7. Keep tube(s) on magnet stand for 1 minute, and remove residual supernatant

First Wash

8. Add 1000 µl of **cfPure Wash Buffer** to lysis/binding tube(s)
9. Resuspend beads by vortexing for 10 seconds or pipetting up and down 6 times
10. Transfer magnetic particle suspension into 1.5 ml micro tube(s) on magnet stand
11. Allow beads to attach to magnet stand for 10-30 seconds
12. Pipette supernatant from 1.5 ml tube(s) and use the supernatant to wash the lysis/binding tube(s)
13. Transfer the rest of the magnetic particles in lysis/binding tube(s) to the 1.5 ml tube(s)
14. Keep tube(s) on magnet stand for 10-30 seconds or until solution is clear
15. Remove as much buffer as possible using a 1000 µl pipette
16. Tap magnet stand on bench 5 times and remove remaining wash buffer with 200 µl pipette
17. Transfer tube(s) to non-magnetic rack and add 1000 µl of **cfPure Wash Buffer**

18. Resuspend beads by vortexing for 20 seconds or pipetting up and down 6 times
19. Centrifuge tube(s) briefly

    *Centrifuge steps are needed when using vortexing to resuspend beads. Only a brief spin is recommended to remove solution from tube(s) lid
20. Place tube(s) on magnet stand for 10-30 seconds
21. Remove as much buffer as possible using a 1000 µl pipette
22. Tap magnet stand on bench 5 times and remove remaining wash buffer with 200 µl pipette

Second Wash

23. Transfer tube(s) to non-magnetic rack and add 1000 µl of **80% EtOH**
24. Resuspend beads by vortexing for 20 seconds or pipetting up and down 6 times
25. Centrifuge tube(s) briefly
26. Place on magnet stand for 10-30 seconds or until solution clears
27. Remove as much buffer as possible using a 1000 µl pipette
28. Tap magnet stand on bench 5 times and remove remaining EtOH with 200 µl pipette
29. Transfer tube(s) to non-magnetic rack and add 1000 µl of **80% EtOH**
30. Resuspend beads by vortexing for 20 seconds or pipetting up and down 6 times
31. Centrifuge tube(s) briefly
32. Place on magnet stand for 10-20 seconds
33. Remove as much EtOH as possible using a 1000 µl pipette and leave cap open
34. Tap magnet stand with tube(s) on bench 5 times
35. Remove remaining EtOH with 200 µl pipette
36. Leave tube(s) open on magnet stand for two minutes and then tap tube(s) on bench 5 times and remove any remaining EtOH with 20 µl pipette
37. Allow magnetic particles to dry for an additional 1-3 minutes

    *Be careful to not over dry or beads may stick to tube(s)

Elution Step

38. Transfer microtube(s) to non-magnetic rack and add desired volume of **cfPure Elution Buffer** and resuspend beads

    Important:: A minimum of 12.5 µl of cfPure Elution Buffer per ml of plasma is recommended to elute DNA to ensure optimal yields
39. Vortex or shake tube(s) vigorously for 5 minutes
40. Centrifuge tube(s) briefly
41. Place tube(s) on magnetic rack for 10 to 30 seconds
42. Transfer elute into a new 1.5 ml tube(s)

Kit Components for 100ml Kit (K5011610)

Item	Cat#	Amount	Storage
1. Lysis/Binding Buffer	K5011610-1	1 x 115 ml	Room Temp
2. Wash Buffer	K5011610-2	2 x 55 ml	Room Temp
3. Elution Buffer	K5011610-3	1 x 6 ml	Room Temp
4. Magnetic Bead Solution	K5011610-4	2 x 1.33 ml	Room Temp

Kit Components for 250ml Kit (K5011625)

Item	Cat#	Amount	Storage
1. Lysis/Binding Buffer	K5011625-1	3 x 95 ml	Room Temp
2. Wash Buffer	K5011625-2	5 x 55 ml	Room Temp
3. Elution Buffer	K5011625-3	1 x 15 ml	Room Temp
4. Magnetic Bead Solution	K5011625-4	5 x 1.33 ml	Room Temp