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# User's Manual and Instructions

## cfPure® Cell-Free DNA Extraction Kit

**Catalog Number:** K5011610, K5011625, K5011625MA

**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
- Fully automatable using KingFisher Flex Purification System and Robotic Liquid Handlers
- Purified DNA is suitable for NGS, PCR, Bisulfite sequencing, etc

**Description**
BioChain's 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 and King Fisher Flex Purification System. This kit may also be applied on Hamilton's Presto system with the appropriate program. 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 3 package sizes for this kit, which contains sufficient reagents for isolating cfDNA from up to 100 ml, 250 ml, or 250 ml's of sample in 5 - 10 ml increments.

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

**Important Notes**

**In general, this cfPure kit performs best with higher yields and complete cfDNA capture when double-spun plasma that is free of contaminating white blood cells is used. However, if the plasma was not double-spun immediately upon collection or there is other gDNA contamination in the plasma, then we strongly recommend using the cfPure V2 kit instead (catalog numbers K5011610-V2, K5011625-V2, and K5011625MA-V2). V2 will minimize the gDNA contamination in the final elution, although it should be noted that some of the cfDNA may also be lost.**

Blood Collection: The cfPure® Cell-Free DNA extraction kit has been optimized for use with samples collected in Streck Cell-Free DNA BCT, EDTA tubes and Acid Dextrose Acid (ACD) tubes.

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.

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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%

**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 BioChain® Technical Service for additional recommendations for high throughput or automated mixing.

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**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. 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 kits (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

**For 250 ml Max kits (K5011625MA)**

- Add 19 ml of fresh 100% ethanol to each bottle of Lysis/Binding Buffer and mix by inverting gently
- Add 60 ml of fresh 100% ethanol to 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.

## Small (0.2 ml to 7.9 ml) Sample Protocol

| Plasma | Lysis/Binding Buffer | Bead Solution* | Tube(s) size |
|---|---|---|---|
| x (x=ml of plasma) | 1.25x | 0.025x | n/a |
| 5 ml | 6.25 ml | 125 µl | 15 ml or 50ml** |
| 7 ml | 8.75 ml | 175 µl | 50 ml |

*** Important:** for quantitative PCR applications, use 0.008x instead (e.g. 40 µl of bead solution for 5 ml plasma and 56 µl of bead solution for 7 ml plasma). The final yield may be ~10-20% lower, but the CT values obtained during qPCR will be reflective of the actual quantities in the reaction. Using 0.025x bead solution will maximize the yield but may result in a ~1-2 CT value delay.

******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 |
| 5 ml | 75 µl | 250 µl |
| 7 ml | 105 µl | 350 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 (or 8 µl: see note under the table on page 3) 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 10 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 10 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 10 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 10 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)

## Large (8 ml to 20 ml) Sample Extraction Protocol

This protocol is optimized for use with samples with volumes of 8 ml or larger.

| Plasma | Lysis/Binding Buffer | Bead Solution* | Tube(s) size |
|---|---|---|---|
| x (x=ml of plasma) | 1.25x | 0.020x | n/a |
| 8 ml | 10.00 ml | 160 µl | 50 ml |
| 10 ml | 12.50 ml | 200 µl | 50 ml |

*** Important:** for quantitative PCR applications, use 0.008x instead (e.g. 40 µl of bead solution for 5 ml plasma and 56 µl of bead solution for 7 ml plasma). The final yield may be ~10-20% lower, but the CT values obtained during qPCR will be reflective of the actual quantities in the reaction. Using 0.020x bead solution will maximize the yield but may result in a ~1-2 CT value delay.

**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 |
| 8 ml | 120 µl | 400 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 appropriate amount of plasma to a 50 ml conical tube(s)
2. Add 1.25 ml of **cfPure Lysis/Binding Buffer** for every 1 ml of plasma used
3. Add 20 µl (or 8 µl: see note under the table on page 6) of **cfPure Magnetic Bead Solution** for every 1 ml of plasma used

   **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 5 to 10 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 2000 µl of **cfPure Wash Buffer** to lysis/binding tube(s)
9. Resuspend beads by vortexing for 20 seconds or by manually swirling tube(s)
10. Place 50 ml tube(s) on to a magnetic stand for 2 to 5 minutes, or until solution clears
11. Using a 1000 ul pipette wash the tube(s) using the supernatant within the tube

    **Important:** Washing the side of the tube(s) will ensure all beads are attached to the magnet. The cap of the tube may also need to be washed if beads have stuck to the cap.
12. Keep 50 ml tube(s) on to a magnetic stand for an additional 2 minutes
13. While keeping the tube(s) on the magnet stand, remove supernatant. Be careful not to remove magnetic particles
14. Transfer tube(s) to non-magnetic rack and add 1000 µl of **cfPure Wash Buffer**
15. Resuspend beads by vortexing for 20 seconds or pipetting up and down 10 times

16. Transfer magnetic particle suspension into 1.5 ml micro tube(s) on a magnet stand
17. Allow beads to attach to magnet stand for 10-30 seconds
18. Pipette supernatant from 1.5 ml tube(s) and use the supernatant to wash the lysis/binding tube(s)
19. Transfer the rest of the magnetic particles in lysis/binding tube(s) to the 1.5 ml tube(s)
20. Resuspend beads by vortexing for 20 seconds or pipetting up and down 10 times
21. 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
22. Place tube(s) on magnet stand for 10-30 seconds or until solution is clear
23. Remove as much buffer as possible using a 1000 µl pipette
24. Tap magnet stand on bench 5 times and remove remaining wash buffer with 200 µl pipette

**Second Wash**

25. Transfer tube(s) to non-magnetic rack and add 1000 µl of **80% EtOH**
26. Resuspend beads by vortexing for 20 seconds or pipetting up and down 10 times
27. Centrifuge tube(s) briefly
28. Place on magnet stand for 10-30 seconds or until solution clears
29. Remove as much buffer as possible using a 1000 µl pipette
30. Tap magnet stand on bench 5 times and remove remaining EtOH with 200 µl pipette
31. Transfer tube(s) to non-magnetic rack and add 1000 µl of **80% EtOH**
32. Resuspend beads by vortexing for 20 seconds or pipetting up and down 10 times
33. Centrifuge tube(s) briefly
34. Place on magnet stand for 10-20 seconds
35. Remove as much EtOH as possible using a 1000 µl pipette and leave cap open
36. Tap magnet stand with tube(s) on bench 5 times
37. Remove remaining EtOH with 200 µl pipette
38. 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
39. Allow magnetic particles to dry for an additional 4 minutes

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

**Elution Step**

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

    **Important:** For optimal yields a minimum of 100 µl of cfPure Elution Buffer should be used.
41. Vortex or shake tube(s) vigorously for 5 minutes
42. Centrifuge tube(s) briefly
43. Place tube(s) on magnetic rack for 10 to 30 seconds
44. Transfer eluate into a new 1.5 ml tube(s)

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# Alternative Low Elution Volume Protocol

The low elution volume protocol is recommended when 50 ul or less of elution when extracting from 10 ml samples.

| Plasma | Lysis/Binding Buffer | Bead Solution | Tube(s) size |
|---|---|---|---|
| 10 ml | 12.50 ml | 75 µl | 50 ml |

**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 |
| 10 ml | 150 µl | 500 µl |

1. Add the 10 ml's of plasma to an appropriately sized tube(s)
2. Add 150 µl of Proteinase K (20 mg/ml)
3. Add 500 µl of 20% SDS solution
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 10 ml of plasma to 50 ml tube(s)
2. Add 12.5 ml of **cfPure Lysis/Binding Buffer** to the tube(s)
3. Add 75 µl of **cfPure Magnetic Bead Solution** to the tube(s)

   **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 5 to 10 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 swirling tube or pipetting up and down 10 times
10. Transfer magnetic particle suspension into 2 ml micro tube(s) on magnet stand
11. Allow beads to attach to magnet stand for 20-30 seconds or until solution clears
12. Pipette supernatant from 2 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 2 ml tube(s)
14. Keep tube(s) on magnet stand for 20-30 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 25 seconds or pipetting up and down 10 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 20-30 seconds or until solution clears
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 10 times
25. Centrifuge tube(s) briefly
26. Place on magnet stand for 20-30 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 10 times
31. Centrifuge tube(s) briefly
32. Place on magnet stand for 20-30 seconds or until solution clears

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 4 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 50 ul of **cfPure Elution Buffer** and resuspend beads
39. Vortex or shake tube(s) vigorously for 5 minutes
40. Centrifuge tube(s) briefly
41. Place tube(s) on magnetic rack for 20 to 30 seconds or until solution clears
42. Transfer eluate into a new 1.5 ml tube(s)

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**Kit Components cfPure cfDNA Extraction, 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 cfPure cfDNA Extraction, 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 |

**Kit Components cfPure MAX cfDNA Extraction, 250ml Kit (K5011625MA)**

| Item | Cat# | Amount | Storage |
|---|---|---|---|
| 1. Lysis/Binding Buffer | K5011625MA-1 | 3 x 95 ml | Room Temp |
| 2. Wash Buffer | K5011625MA-2 | 1 x 65 ml | Room Temp |
| 3. Elution Buffer | K5011625MA-3 | 1 x 15 ml | Room Temp |
| 4. Magnetic Bead Solution | K5011625MA-4 | 5 x 1.33 ml | Room Temp |

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# cfPure™ Cell-Free DNA Extraction Kit

Isolation of cfDNA from 1 - 5 ml of sample using KingFisher™ Flex Magnetic Processor 24DW

**Catalog Number:** K5011610, K5011625

**Product Description**

Biochain's new cfPure Cell-Free DNA Extraction Kit has been designed to isolate circulating DNA from human plasma and serum. The cfPure utilizes Silica coated magnetic bead technology allowing for scalability and easy automation. The cfPure Cell-Free DNA extraction Kit can be used to isolate cfDNA from up to 24 samples of 1 - 5 ml of plasma or serum using the KingFisher™ Flex Magnetic Processor with 24 Deep Well Head. This guide describes the use of the cfPure kit with the KingFisher™ Flex Magnetic Processor 24DW to process samples of 1 - 5 ml .

**Kit Contents and Storage**

CfPure cfDNA Extraction Kit , 100ml (K5011610)

| Item | Amount | Storage |
|---|---|---|
| cfPure Lysis/Binding Buffer | 1 x 115 ml | Room Temp. |
| cfPure Wash Buffer | 2 x 55 ml | |
| cfPure Elution Buffer | 1 x 6 ml | |
| cfPure Magnetic Bead Solution | 2 x 1.33 ml | |

CfPure cfDNA Extraction Kit , 250ml (K5011625)

| Item | Amount | Storage |
|---|---|---|
| cfPure Lysis/Binding Buffer | 3 x 95 ml | Room Temp. |
| cfPure Wash Buffer | 5 x 55 ml | |
| cfPure Elution Buffer | 1 x 15 ml | |
| cfPure Magnetic Bead Solution | 5 x 1.33 ml | |

**Equipment and Reagents to be Supplied by User**

| Item | Source |
|---|---|
| **Equipment** | |
| Multi-channel micropipettors | Any |
| Adjustable Micropipettors | Any |
| Vortexor | Any |
| **Magnetic Particle Processor** | |
| KingFisher™ Flex Magnetic Particle Processor | Thermofisher 5400630 |
| **Magnetic Head** | |
| 24 Deep-Well Plates for KingFisher™ Flex Magnetic Particle Processor | Thermofisher 24074440 |
| **Deep-Well Plates** | |
| KingFisher™ Flex 24 deep well plate, sterile | Thermofisher 95040490 |
| **Tip Combs** | |
| King Fisher Flex 24 Deep Well Tip Comb and Plate | Thermofisher 97002610 |

| Item | Source |
|---|---|
| **Consumables** | |
| Aerosol-resistant pipette tips | Any |
| Nonstick, RNase-free Microfuge tubes (1.5ml) | Any |
| MicroAmp™ Clear Adhesive Film | Any |
| Reagent Reservoirs | Any |
| **Reagents** | |
| Ethanol, 200 proof (Absolute) | Any |
| SDS, 20% Solution (Only required for Proteinase K treament) | Any |
| Proteinase K solution (20mg/ml) (Only required for Proteinase K treament) | BioChain Z5050002 |

**Download KingFisher™ Flex Program**

**1**.On cfPure Webpage scroll down to Manual Section.
**2.** Click cfPure_4-5ml_Flex and/or cfPure_1-2ml_Flex to download program to your computer
**3.** Refer to KingFisher™ Flex manual for instructions for installing program on the instrument

**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.
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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**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. 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

**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=µl of plasma) | 0.015 x | 0.050 x |
| 1 ml | 15 l | 50 µl |
| 2 ml | 30 µl | 100 µl |
| 4 ml | 60 µl | 200 µl |
| 5 ml | 75 µl | 250 µl |

8. Add the appropriate amount of plasma to an appropriately sized tube(s)
9. Add 15 µl of Proteinase K (20 mg/ml) for every 1 ml of plasma used
10. Add 50 µl of 20% SDS solution for every 1 ml of plasma used
11. Mix by inverting gently 5 times
12. Incubate at 60°C for 20 minutes
13. After incubation place tube(s) on ice for 5 minutes to cool tube(s) to room temperature

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**Plate Set up for 1 or 2 ml samples**

Set up 24 Deep Well Plates by adding appropriate reagents according to table below

| Plate ID | Plate Type | Plate Position | Reagent | Volume per well | |
|---|---|---|---|---|---|
| | | | | 1 ml | 2 ml |
| Lysis/Binding Plate | 24 DW Plate | 1 | cfPure Lysis/Binding Buffer | 1.25 ml | 2.5 ml |
| | | | cfPure Magnetic Bead Solution | 25 µl* | 50 µl* |
| Wash Plate 1 | 24 DW Plate | 2 | cfPure Wash Buffer | 1 ml | |
| Wash Plate 2 | 24 DW Plate | 3 | cfPure Wash Buffer | 1 ml | |
| Wash Plate 3 | 24 DW Plate | 4 | 80% Ethanol | 2 ml | |
| Wash Plate 4 | 24 DW Plate | 5 | 80% Ethanol | 1 ml | |
| Elution Plate | 24 DW Plate | 6 | cfPure Elution Buffer | 50 - 100 µl | |
| Tip Comb | 24 DW Plate | 7 | Place a 24 Deep-Well Tip Comb in Plate | | |

*** Important:** for quantitative PCR applications, use 8 µl or 16 µl of beads for 1 ml or 2 ml of plasma, respectively. The final yield may be ~10-20% lower, but the CT values obtained during qPCR will be reflective of the actual quantities in the reaction. Using the bead volumes in the above table will maximize the yield but may result in a ~1-2 CT value delay.

**Plate Set up for 4 or 5 ml samples**

Set up 24 Deep Well Plates by adding appropriate reagents according to table below

| Plate ID | Plate Type | Plate Position | Reagent | Volume per well | |
|---|---|---|---|---|---|
| | | | | 4 ml | 5 ml |
| Lysis/Binding Plate 1 | 24 DW Plate | 1 | cfPure Lysis/Binding Buffer | 2.5 ml | 3.125 ml |
| | | | cfPure Magnetic Bead Solution | 50 µl* | 62.5 µl* |
| Lysis/Binding Plate 2 | 24 DW Plate | 2 | cfPure Lysis/Binding Buffer | 2.5 ml | 3.125 ml |
| | | | cfPure Magnetic Bead Solution | 50 µl* | 62.5 µl* |
| Wash Plate 1 | 24 DW Plate | 3 | cfPure Wash Buffer | 1 ml | |
| Wash Plate 2 | 24 DW Plate | 4 | cfPure Wash Buffer | 1 ml | |
| Wash Plate 3 | 24 DW Plate | 5 | 80% Ethanol | 2 ml | |
| Wash Plate 4 | 24 DW Plate | 6 | 80% Ethanol | 1 ml | |
| Elution Plate | 24 DW Plate | 7 | cfPure Elution Buffer | 50 - 100 µl | |
| Tip Comb | 24 DW Plate | 8 | Place a 24 Deep-Well Tip Comb in Plate | | |

*** Important:** for quantitative PCR applications, use 16 µl or 20 µl of beads in each Lysis/Binding plate above for 4 ml or 5 ml of plasma, respectively. The final yield may be ~10-20% lower, but the CT values obtained during qPCR will be reflective of the actual quantities in the reaction. Using the bead volumes in the above table will maximize the yield but may result in a ~1-2 CT value delay.

- Gently shake Lysis/Binding Plate(s) to mix the reagents
- If extracting cfDNA from a 2 ml sample add enitre sample to a well on Lysis/Binding Plate
- If extracting cfDNA from a 4 or 5 ml sample add half of sample to a well on Lysis/Binding Plate 1 and the other half of sample to the same well on Lysis/Binding Plate 2

**Instrument Set up**

- Place 24 Deep-Well magnetic head on to machine according the manuals protocol
- Select cfPure_4-5ml_Flex on the instrument for 4 or 5 ml extraction or cfPure_1-2ml_Flex for 1 or 2 ml extractions
- Start the run and follow on screen prompts to load processing plates in their respective positions
- At the end of the run remove elution plate from machine and cover plate or transfer eluate to new tubes

Isolated cfDNA is ready for immediate use or can be stored at -20°C

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# cfPure™ Cell-Free DNA Extraction Kit

Isolation of cfDNA using KingFisher™ Flex Magnetic Processor 96DW

**Catalog Number:** K5011610, K5011625

**Product Description**

Biochain's new cfPure Cell-Free DNA Extraction Kit has been designed to isolate circulating DNA from human plasma and serum. The cfPure utilizes Silica coated magnetic bead technology allowing for scalability and easy automation. The cfPure Cell-Free DNA extraction Kit can be used to isolate cfDNA from up to 96 samples of 600 µl of plasma or serum using the KingFisher™ Flex Magnetic Processor with 96 Deep Well Head. This guide describes the use of the cfPure kit with the KingFisher™ Flex Magnetic Processor 96DW to process samples of 1000 µl or less.

**Kit Contents and Storage**

CfPure cfDNA Extraction Kit , 100ml (K5011610)

| Item | Amount | Storage |
|---|---|---|
| cfPure Lysis/Binding Buffer | 1 x 115 ml | Room Temp. |
| cfPure Wash Buffer | 2 x 55 ml | |
| cfPure Elution Buffer | 1 x 6 ml | |
| cfPure Magnetic Bead Solution | 2 x 1.33 ml | |

CfPure cfDNA Extraction Kit , 250ml (K5011625)

| Item | Amount | Storage |
|---|---|---|
| cfPure Lysis/Binding Buffer | 3 x 95 ml | Room Temp. |
| cfPure Wash Buffer | 5 x 55 ml | |
| cfPure Elution Buffer | 1 x 15 ml | |
| cfPure Magnetic Bead Solution | 5 x 1.33 ml | |

**Equipment and Reagents to be Supplied by User**

| Item | Source |
|---|---|
| **Equipment** | |
| Multi-channel micropipettors | Any |
| Adjustable Micropipettors | Any |
| Vortexor | Any |
| **Magnetic Particle Processor** | |
| KingFisher™ Flex Magnetic Particle Processor 96DW | Thermofisher 5400630 |
| **Deep-Well Plates** | |
| 96 Deep-Well Plates for KingFisher™ Flex Magnetic Particle Processor | Thermofisher 95040460 |
| **Standard Plates** | |
| 96 Standard Plates for KingFisher™ Flex Magnetic Particle Processor | Thermofisher 97002540 |
| **Tip Combs** | |
| 96 Deep-Well Tip Combs for KingFisher™ Flex Magnetic Particle | Thermofisher 97002534 |

| Item | Source |
|---|---|
| **Consumables** | |
| Aerosol-resistant pipette tips | Any |
| Nonstick, RNase-free Microfuge tubes (1.5ml) | Any |
| MicroAmp™ Clear Adhesive Film | Any |
| Reagent Reservoirs | Any |
| **Reagents** | |
| Ethanol, 200 proof (Absolute) | Any |
| SDS, 20% Solution (Only required for Proteinase K treament) | Any |
| Proteinase K solution (20mg/ml) (Only required for Proteinase K treament) | BioChain Z5050002 |

**Download KingFisher™ Flex Program**

**1**.On cfPure Webpage scroll down to Manual Section.
**2.** Click cfPure_600ul_Flex to download program to your computer
**3.** Refer to KingFisher™ Flex manual for instructions for installing program on the instrument

**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.
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%

**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. 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

**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=µl of plasma) | 0.015 x | 0.050 x |
| 600 µl | 9.0 µl | 30 µl |
| 1000 µl | 15 µl | 50 µl |

14. Add the appropriate amount of plasma to an appropriately sized tube(s)
15. Add 15 µl of Proteinase K (20 mg/ml) for every 1 ml of plasma used
16. Add 50 µl of 20% SDS solution for every 1 ml of plasma used
17. Mix by inverting gently 5 times
18. Incubate at 60°C for 20 minutes
19. After incubation place tube(s) on ice for 5 minutes to cool tube(s) to room temperature

**Plate Set up**

Set up 96 Plates by adding appropriate reagents according to table below

| ` | Plate Type | Plate Position on Instrument | Reagent | Volume per well | |
|---|---|---|---|---|---|
| | | | | 600 ul | 1000 ul |
| Lysis/Binding Plate 1 | 96 Deep-Well Plate | 1 | cfPure Lysis/Binding Buffer | 375 µl | 625 µl |
| | | | cfPure Magnetic Bead Solution | 7.5 µl* | 12.5 µl* |
| Lysis/Binding Plate 2 | 96 Deep-Well Plate | 2 | cfPure Lysis/Binding Buffer | 375 µl | 625 µl |
| | | | cfPure Magnetic Bead Solution | 7.5 µl* | 12.5 µl* |
| Wash Plate 1 | 96 Deep-Well Plate | 3 | cfPure Wash Buffer | 1 ml | 1 ml |
| Wash Plate 2 | 96 Deep-Well Plate | 4 | cfPure Wash Buffer | 1 ml | 1 ml |
| Wash Plate 3 | 96 Deep-Well Plate | 5 | 80% Ethanol | 1 ml | 1 ml |
| Wash Plate 4 | 96 Deep-Well Plate | 6 | 80% Ethanol | 500 µl | 500 µl |
| Elution Plate | 96 Standard Plate | 7 | cfPure Elution Buffer | 30 - 50 µl | 30 - 50 µl |
| Tip Comb | 96 Deep-Well Plate | 8 | | Place a 96 Deep-Well Tip Comb in Plate | |

*** Important:** for quantitative PCR applications, use 2.4 µl or 4 µl of beads in each Lysis/Binding plate above for 600 µl or 1000 µl of plasma, respectively. The final yield may be ~10-20% lower, but the CT values obtained during qPCR will be reflective of the actual quantities in the reaction. Using the bead volumes in the above table will maximize the yield but may result in a ~1-2 CT value delay.

- Gently shake Lysis/Binding Plate 1 and 2 to mix the reagents
- Add half of plasma sample to the same wells of Lysis/Binding Plate 1 and 2

**Instrument Set up**

- Place 96 well Deep-Well magnetic head on to machine, and select cfPure_1ml_96_Flex on the instrument
- Start the run and follow on screen prompts to load processing plates in their respective positions
- At the end of the run remove elution plate and cover immediately

Isolated cfDNA is ready for immediate use