MAGBIO

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cfKapture Kit - DX (200-400 µL)

Isolation of cfDNA from Plasma

Catalog Nos. CFK-DI10-400ULE, CFK-DI50-400ULE
Manual Revision 0
WI-72-150

- Isolation of circulating cell-free DNA from plasma
- Magnetic bead-based chemistry

Instructions For Use

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For *in vitro* diagnostic procedures.

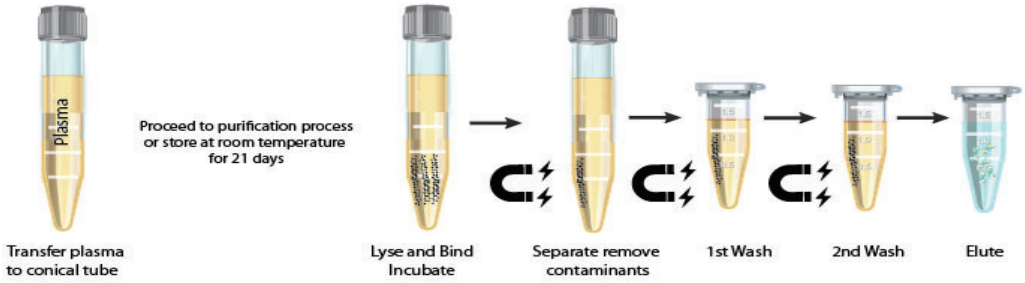
Information in this document is subject to change without notice.

Product Description

The cfKapture Kit - DX is used for purification of circulating, cell-free nucleic acids from plasma. It's designed for the purification of circulating cell-free DNA (ccfDNA) from maternal and cancer patient's blood plasma. The kit can isolate and concentrate cell-free DNA from fresh or frozen samples. It eliminates high molecular weight DNA, leaving behind cfDNA. The isolated cfDNA can be directly used for real time-PCR and DNA library preparation suitable for next generation sequencing. This protocol can be used in manual procedures as well as a guideline for adapting the kit to automated instruments.

This product is intended to be used by qualified and trained laboratory professionals only.

Process



Kit Contents and Storage

cfKapture Kit - DX (200-400 µL) Catalog No.*	CFK-DI10-400ULE	CFK-DI50-400ULE	Storage
Number of Preps	10	50	
CFL Buffer	5 mL	22 mL	15-25°C
CFW1 Buffer [1]	8 mL	40 mL	15-25°C
CFW2 Buffer [1]	5 mL	25 mL	15-25°C
Elution Buffer	600 µL	3 mL	15-25°C
Pro K Solution [2]	220 µL	1.1 mL	2-8°C
MAG-CFB Particles [3]	220 µL	1.1 mL	2-8°C

[1] Ethanol must be added prior to use. See Preparation of Reagents section.
*Once opened, reagents are usable until the expiration date on the product label. Be sure to close the lid firmly before storing reagents for later use.

Shipping and Storage

- [2] Pro K Solution comes in a ready to use solution. Ships at room temperature. Store at 2-8°C.
- [3]MAG-CFB Particles ship at room temperature. Store at 2-8°C.

Safety Information

Any consumables, including plates, tubes, etc., used to process samples with infectious or microbial hazards should be disposed of in an appropriate biohazard waste bin. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). The SDS can be downloaded from the "Product Documents" tab when viewing this product at www.magbiogenomics.com.

Preparation of Reagents

Prepare the following components for each kit before use.

Equipment and Reagents to Be Supplied by the User

☐ Ethanol (100%)

Catalog No.	Component	Add 100% Ethanol	Storage
CFK-DI10-400ULE	CFW1 Buffer	10 mL*	15-25°C
	CFW2 Buffer	13 mL*	15-25°C

Catalog No.	Component	Add 100% Ethanol	Storage
CFK-DI50-400ULE	CFW1 Buffer	50 mL*	15-25°C
	CFW2 Buffer	65 mL*	15-25°C

**Ensure bottle/tube lid Is closed tightly when preparing and storing reagents.*

Protocol: 200 µL sample volume (1.5/2.0 mL tube format)

Equipment and Reagents to Be Supplied by the User

- ☐ RNase and DNase-free 1.5 mL or 2.0 mL microcentrifuge tubes
- ☐ Magnetic separation device for a 1.5 mL/2.0 mL microcentrifuge tube (see page 8)
- ☐ 100% Ethanol
- ☐ Vortex
- ☐ Tube rotator
- ☐ Water bath, incubator, or heat block capable of 60°C

Things to do Before Starting

- ☐ Prepare all reagents according to the instructions on page 2
- ☐ Preheat water bath, incubator, or heat block to 60°C
- ☐ Warm Elution Buffer to 60°C

Protocol

⚠ Bring the **MAG-CFB Particles** to room temperature for at least 30 min before use.

1. Transfer 200 µL of plasma to a 1.5/2.0 mL microcentrifuge tube.

2. Add 20 µL of **Pro K Solution** and mix well by vortexing at maximum speed for 20 seconds.

3. Incubate the sample at 60°C for 10 min in a water bath. Mix by inverting the tube once during incubation.

4. Add 200 µL of **CFL Buffer** and mix well by vortexing at maximum speed for 60 seconds. Incubate the sample at room temperature for 5 min.

5. Add 150 µL of 100% Ethanol and 20 µL of **MAG-CFB Particles**. Mix immediately by vortexing at maximum speed for 20 seconds.

   ⚠ *Complete resuspension of the **MAG-CFB Particles** is crucial for obtaining purity.*

6. Incubate the sample tube on a tube rotator for 20 min at room temperature at 10 rpm. Adjust the rotator angle to approximately 45 degrees for better mixing.

7. Remove the tube from rotator and place on a compatible 1.5 mL magnetic separation device to magnetize the **MAG-CFB Particles** for 5 min or until the magnetic particles are completely cleared from the solution.

8. With the tube on the magnetic separation device, remove and discard the cleared supernatant by pipetting.

   ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

9. Remove the sample tube from the magnetic separation device.

10. Add 800 µL of **CFW1 Buffer** and resuspend the magnetic particles by vortexing at maximum speed for 1 minute or by pipetting up and down 10 times.

11. Place the 1.5 mL sample tube on a compatible 1.5 mL magnetic separation device to magnetize the **MAG-CFB Particles** at room temperature for 5 min or until the magnetic particles are completely cleared from the solution.

12. Remove and discard the cleared supernatant.

    ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

13. Remove the sample from the magnetic separation device and repeat the wash by adding 800 µL of **CFW1 Buffer** and resuspend the magnetic particles by vortexing at maximum speed for 1 minute or by pipetting up and down 10 times.

14. Place the 1.5 mL sample tube back on the magnetic separation device to magnetize the **MAG-CFB Particles** at room temperature for 5 min or until the magnetic particles are completely cleared from the solution.

15. With the sample still on the magnetic separation device, remove and discard the cleared supernatant.

    ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

16. Remove the sample tube from the magnetic separation device.

17. Add 800 µL of **CFW2 Buffer** and resuspend the magnetic particles by vortexing at maximum speed for 1 minute or by pipetting up and down 10 times.

18. Place the 1.5 mL sample tube on the magnetic separation device to magnetize the **MAG-CFB Particles** at room temperature for 5 min or until the magnetic particles are completely cleared from the solution.

19. With the sample still on the magnetic separation device, remove and discard the cleared supernatant.

    ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

20. Repeat Steps 16-19 for a second **CFW2 Buffer** wash.

21. With the sample tube still on the magnetic separation device, air dry the **MAG-CFB Particles** for 10 min. Remove any residual liquid with a pipette.

    ⚠ *It is critical to completely remove all liquid from the tube.*

22. Remove the sample tube containing the **MAG-CFB Particles** from the magnetic separation device.

23. Add 30-50 µL of **Elution Buffer** and completely resuspend the **MAG-CFB Particles** by vortexing at maximum speed for 10 seconds.

    ⚠ *Heat **Elution Buffer** at 60°C to improve the yield.*

24. Incubate at room temperature for 10 min.

25. Place the tubes back on the magnetic separation device and wait 5 min or until the magnetic particles are completely cleared from the **Elution Buffer**.

26. Transfer the clear supernatant containing the ccfDNA to a new 1.5 mL microcentrifuge tube and store at -20°C.

Protocol: 400 µL sample volume (1.5 mL/2.0 tube format)

Equipment and Reagents to Be Supplied by the User

- ☐ RNase and DNase-free 1.5 mL or 2.0 mL microcentrifuge tubes
- ☐ Magnetic separation device for 1.5 mL/2.0 mL microcentrifuge tube and (see page 8)
- ☐ 100% Ethanol
- ☐ Vortex
- ☐ Tube rotator
- ☐ Water bath, incubator, or heat block capable of 60°C

Things to do Before Starting

- ☐ Prepare all reagents according to the instructions on page 2
- ☐ Preheat water bath, incubator, or heat block to 60°C
- ☐ Warm Elution Buffer to 60°C

Protocol

⚠ Bring the **MAG-CFB Particles** to room temperature for at least 30 min before use.

1. Transfer 400 µL of plasma sample to a 1.5/2.0 mL microcentrifuge tube.

2. Add 20 µL of **Pro K Solution** and mix well by vortexing at maximum speed for 20 seconds.

3. Incubate the sample at 60°C for 10 min in a water bath. Mix by inverting the tube once during incubation.

4. Add 400 µL of **CFL Buffer** and mix well by vortexing at maximum speed for 60 seconds. Incubate the sample at room temperature for 10 min.

5. Add 300 µL of 100% Ethanol and 20 µL of **MAG-CFB Particles**. Mix immediately by vortexing at maximum speed for 20 seconds.

   ⚠ *Complete resuspension of the **MAG-CFB Particles** is crucial for obtaining purity.*

6. Incubate sample tube on a tube rotator for 20 min at room temperature at 10 rpm. Adjust the rotator angle to approximately 45 degrees for better mixing.

7. Remove the tube from rotator and place on a compatible 2 mL magnetic separation device to magnetize the **MAG-CFB Particles** for 5 min or until the magnetic particles are completely cleared from the solution.

8. With the tube on the magnetic separation device, remove and discard the cleared supernatant by pipetting.

   ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

9. Remove the sample tube from the magnetic separation device.

10. Add 800 µL of **CFW1 Buffer** and resuspend the magnetic particles by vortexing at maximum speed for 1 minute or by pipetting up and down 10 times.

11. Place the 2 mL sample tube on a compatible 2 mL magnetic separation device to magnetize the **MAG-CFB Particles** at room temperature for 5 min or until the magnetic particles are completely cleared from the solution.

12. Remove and discard the cleared supernatant.

    ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

13. Remove the sample off the magnetic separation device and repeat the wash by adding 800 µL of **CFW1 Buffer** and resuspend the magnetic particles by vortexing at maximum speed for 1 minute or by pipetting up and down 10 times.

14. Place the 2 mL sample tube back on the magnetic separation device to magnetize the **MAG-CFB Particles** at room temperature for 5 min or until the magnetic particles are completely cleared from the solution.

15. Remove and discard the cleared supernatant.

    ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

16. Remove the sample tube from the magnetic separation device.

17. Add 800 µL of **CFW2 Buffer** and resuspend the magnetic particles by vortexing at maximum speed for 1 minute or by pipetting up and down 10 times.

18. Place the 2 mL sample tube on the magnetic separation device to magnetize the **MAG-CFB Particles** at room temperature for 5 min or until the magnetic particles are completely cleared from the solution.

19. With the sample still on the magnetic separation device, remove and discard the cleared supernatant.

    ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

20. Repeat Steps 16-19 for a second **CFW2 Buffer** wash.

21. With the sample tube on the magnetic separation device, air dry the **MAG-CFB Particles** for 10 min. Remove any residual liquid with a pipette.

    ⚠ *It is critical to completely remove all liquid from the tube.*

22. Remove the sample tube containing the **MAG-CFB Particles** from the magnetic separation device.

23. Add 30-50 µL of **Elution Buffer** and completely resuspend the **MAG-CFB Particles** by vortexing at maximum speed for 10 seconds.

    ⚠ *Heat **Elution Buffer** at 60°C to improve the yield.*

24. Incubate at room temperature for 10 min.

25. Place the tubes back on the magnetic separation device and wait 5 min or until the magnetic particles are completely cleared from the **Elution Buffer**.

26. Transfer the clear supernatant containing the ccfDNA to a new 1.5 mL microcentrifuge tube and store at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via:
Phone: US/Canada, +1 301-302-0144. Europe, +49 7250 33 13 403
Email: US/Canada, support@magbiogenomics.com. Europe, Info.europe@magbiogenomics.com

Symptoms	Possible Causes	Comments
Low yield of cfDNA	Incomplete resuspension of MAG-CFB Particles	Resuspend the MAG-CFB Particles by vortexing vigorously before use
	Loss of MAG-CFB Particles during operation	Avoid disturbing the MAG-CFB Particles during aspiration of supernatant
	Ethanol is not added into CFW1 Buffer or CFW2 Buffer	Add absolute 100% Ethanol to the CFW1 Buffer and CFW2 Buffer (see page 2 for instructions)
MAG-CFB Particles do not completely clear from the solution	Magnetizing time too short	Increase collection time on the magnet
Problems in downstream applications	Insufficient cfDNA in starting material	Use more starting material
	Ethanol carry-over	Dry the MAG-CFB Particles completely before elution
Carryover of MAG-CFB Particles	The eluate has particles and is not fully clear	Increase magnetization time. If small amount of carryover, place eluted sample on a magnetic separation device and perform an additional 5 min magnetization

Ordering Information

cfKapture Kit - DX

Catalog No.	Product	Description	Preps
CFK-DI50-400ULE	cfKapture Kit - DX (200-400 µL, 50 preps)	Purification of cell-free DNA (cfDNA) from 200-400 µL plasma	50
CFK-DI50-2MLE	cfKapture Kit - DX (1-2 mL, 50 preps)	Purification of cell-free DNA (cfDNA) from 1-2 mL plasma	50
CFK-DI50-5MLE	cfKapture Kit - DX (3-5 mL, 50 preps)	Purification of cell-free DNA (cfDNA) from 3-5 mL plasma	50

Related Products

HighPrep PCR - DX

Catalog No.	Product
AC-60005E	HighPrep PCR - DX (5 mL)
AC-60050E	HighPrep PCR - DX (50 mL)
AC-60250E	HighPrep PCR - DX (250 mL)
AC-60500E	HighPrep PCR - DX (500 mL)

Magnetic Separation Devices

Catalog No.	Product
MYMAG-96	Handheld Magnetic Separation Device (96 well microplate format)
MYMAG-96X	Magnetic Separation Device (96 well ring format)
MBMS-12	MagStrip Magnet Stand (1.5 mL x 12)
MBMS-31550	15 mL and 50 mL Magnetic Stand Combo (3 x 15 mL and 3 x 50 mL)

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