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cfKapture™ 21 Kit (3-5 ml)

Manual Revision v1.2

Catalog Nos. CFK-D5-5ML , CFK-D50-5ML

Isolation kit for circulating cell-free DNA from stabilized plasma

PROTOCOL

Contents

For Research Use Only. Not for use in diagnostic procedures.

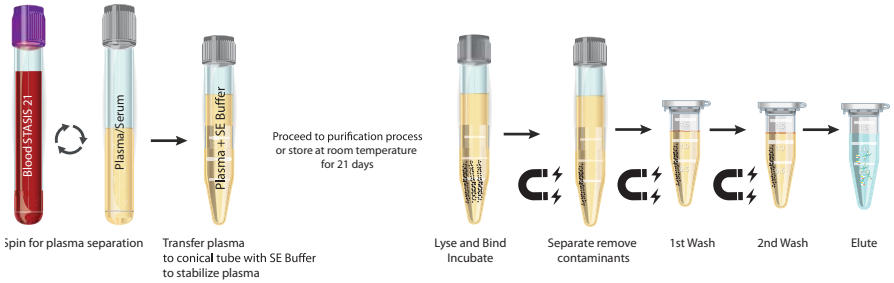
Information in this document is subject to change without notice.

Product Description

cfKapture™ 21 kit is a system for purification of circulating, cell free nucleic acids from plasma. It is designed for the purification of circulating cell free DNA(cfDNA) from maternal and cancer patient's whole blood. cfKapture 21 system includes all necessary reagents from the stabilization of separated plasma to the purification of ccfDNA.

The long term room temperature stabilization of plasma during storage or transport is achieved by mixing the plasma sample with our SE buffer allowing for up to 21 day room temperature stabilization of plasma samples. This functionality offers to samples processing laboratories, time flexibility for optimizing their operational efficiency as well as outreach for receiving samples at local and international levels. The isolated cfDNA can be directly used for real time-PCR and DNA library preparation suitable for next generation sequencing. **This kit is designed for research purposes only.**

Process



Kit Contents and Storage

cfKapture 21 Kit Catalog No.	CFK-D5-5ML	CFK-D50-5ML	STORAGE
Number of Preps	**5**	**50**	
SE Buffer	11 ml	102 ml	15-25°C
CFL Buffer	36 ml	360 ml	15-25°C
DF Solution	60 µl	520 µl	2-8°C
CFW1 Buffer [1]	4 ml	40 ml	15-25°C
CFW2 Buffer [1]	2.5 ml	25 ml	15-25°C
Elution Buffer	550 µl	5.5 ml	15-25°C
Pro K Solution [2]	800 µl	8 ml	2-8°C
MAG-CFB Particles	110 µl	1.1 ml	2-8°C

[1] Ethanol must be added prior to use. See Preparation of Reagents

Stability

All components are stable for 12 months when stored accordingly.

- Pro K Solution comes in a ready to use solution. Component is stable for 1 year when stored at 15-25°C. For storage longer than 1 year, storage at 2-8°C is recommended.
- During shipment or storage in cool ambient conditions, precipitates may form in some buffers. Dissolve such deposits by warming the solution at 37°C and then gently shaking the buffer.

cfKapture 21 - Cell Free DNA Isolation From Stabilized Plasma

MagBio Genomics, Inc.
US/Canada: 1-301-302-0144 | Web: www.magbiogenomics.com | E-mail: info@magbiogenomics.com

Ordering Information

Catalog No.	Product	Description	Preps
CFK-D10-400UL	cfKapture 21 Kit (200-400µl) 10 preps	Purification of cell-free DNA (cfDNA) from 200-400 µl STABILIZED plasma	10
CFK-D5-2ML	cfKapture 21 Kit (1-2ml) 5 preps	Purification of cell-free DNA (cfDNA) from 1-2 ml STABILIZED plasma	5
CFK-D5-5ML	cfKapture 21 Kit (3-5ml) 5 preps	Purification of cell-free DNA (cfDNA) from 3-5 ml STABILIZED plasma	5
CFK-D50-400UL	cfKapture 21 Kit (200-400µl)(50 preps)	Purification of cell-free DNA (cfDNA) from 200-400 µl STABILIZED plasma	50
CFK-D50-2ML	cfKapture 21 Kit (1-2ml) 50 preps	Purification of cell-free DNA (cfDNA) from 1-2 ml STABILIZED plasma	50
CFK-D50-5ML	cfKapture 21 Kit (3-5 ml) 50 preps	Purification of cell-free DNA (cfDNA) from 3-5 ml STABILIZED plasma	50

Magnetic Separation Devices

Catalog No.	Description
MBMS-31550	15ml and 50ml magnetic stand combo. (3x15ml and 3x50ml)
MBMS-10	MagStip magnetic stand (1.5mL x 10)

Whole blood stabilization tubes

Catalog No.	Product	Description
BS21-CF10-100	Blood STASIS 21-ccfDNA 9 mL (100)	100 tubes: 1 ml Additive, 8 ml blood draw volume
BS21-CF6-100	Blood STASIS 21-ccfDNA 6 mL (100)	100 tubes: 0.6 ml Additive, 5.4 ml blood draw volume
BS21-CF3-200	Blood STASIS 21-ccfDNA 3 mL (200)	200 tubes: 0.35 ml Additive, 2.65 ml blood draw volume

cfKapture 21 - Cell Free DNA Isolation From Stabilized Plasma

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). MSDS can be downloaded from the "Product Resource" tab when viewing the product kit.

Equipments Required

Catalog No.	Description
MBMS-31550	Magnetic Separation Device for 15ml tubes
MBMS-10	Magnetic Separation Device for 2ml microcentrifuge tube

Preparation of Reagents

Prepare the following components for each kit before use:

Catalog No.	Component	Add 100% Ethanol	Storage
CFK-D5-5ML	CFW1 Buffer	5 ml	15-25°C
	CFW2 Buffer	6.5 ml	15-25°C
Components are stable for 1 year when stored closed at room temperature			

Catalog No.	Component	Add 100% Ethanol	Storage
CFK-D50-5ML	CFW1 Buffer	50 ml	15-25°C
	CFW2 Buffer	65 ml	15-25°C
Components are stable for 1 year when stored closed at room temperature			

MagBio Genomics, Inc.
US/Canada: 1-301-302-0144 | Web: www.magbiogenomics.com | E-mail: info@magbiogenomics.com

cfKapture™ 21 Kit : 3 ml plasma sample

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) from each product supplier.

- ☐ Nuclease-free 1.5 ml microcentrifuge tubes
- ☐ 15 ml conical tubes
- ☐ Magnetic separation device for 1.5 ml microcentrifuge tube (CAT# MBMS-10)
- ☐ Magnetic separation device for 15 ml conical tubes. (CAT# MBMS-31550)
- ☐ 100% Ethanol
- ☐ Vortex
- ☐ Tube rotator
- ☐ Water bath, incubator or heat block capable of 60°C

Things to do before starting

- ☐ Prepare CFW1 Buffer, CFW2 Buffer according to the "Preparation of Reagents" section on page 2
- ☐ Perheat and warm water bath, incubator or heat block to 60°C
- ☐ Warm Elution Buffer to 60°C

Preparing serum/plasma for stabilization

1. **Add 2ml of SE Buffer to a 15ml conical tube.**
   This tube will be used to stabilize the separated plasma from whole blood samples outlined below. 2mL of SE Buffer can stabilize up to 5 mL of plasma.

2. **Prepare serum/plasma from whole blood:**

⚠ **If processing 9-10ml whole blood from a Blood STASIS™ 21 tube (CAT# BS21-CF10-100), centrifugation steps can be performed at room temperature. If not processing from a Blood STASIS™ 21 tube, all centriguation steps need to be performed at 4°C.**

   a. **Centrifuge whole blood samples at 2,000 g at room temperature.**

   b. **Transfer the plasma to a new 15 ml conical tube.**

   c. **Centrifuge the plasma sample again at 16,000 g for 10 min at room temperature to remove any residual blood and cell debris.**

   d. **Transfer 4-5 ml of clear plasma to the conical tube containing SE Buffer from step 1 to stabiize the plasma.**

   e. **Stabilized plasma can be stored at room temperature for 21 days or proceed to isolation process.**

Troubleshooting guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via:
Phone: 1-855-262-4246 (in US), outside US, 1-301-302-0144
Email: support@magbiogenomics.com

Symptoms	Possible Causes	Comments

18. **Remove and discard the cleared supernatant.** Do not disturb the attracted beads while aspirating the supernatant.

19. **Remove the sample tube off the magnetic separation device.**

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

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

22. **Remove and discard the cleared supernatant.** Do not disturb the attracted beads while aspirating the supernatant.

23. **Repeat Steps 19-22 for a second CFW2 Buffer wash step.**

24. **With the sample tube on the magnetic separation device, air dry the MAG-CFB Particles for 10 minutes. Remove any residual liquid with a pipette.**
Note: It is critical to completely remove all liquid from the tube.

Elution Steps

25. **Remove the sample tube containing the MAG-CFB Particles off the magnetic separation device.**

26. **Add 50-100 µl Elution Buffer and completely resuspend the MAG-CFB Particles by vortexing at maximum speed for 10 seconds.**
Note: Heat Elution Buffer at 60°C to improve yield.

27. **Incubate at room temperature for 10 minutes.**

28. **Place the tubes back on the magnetic separation device and wait 5 minutes or until the magnetic particles are completely cleared from elution buffer.**

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

Binding Steps

3. **Transfer 4.2 ml (plasma+SE Buffer) from the stabilized plasma sample to a new 15 ml conical tube.**

4. **Add 100 µl Pro K Solution, and mix well by vortexing at maximum speed for 20 seconds.**

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

6. **Add 4.2 ml CFL Buffer and mix well by vortexing at maximum speed for 60 seconds, and incubate sample at room temperature for 10 minutes.**

7. **Add 3.15 ml 100% ethanol and 20 µl MAG-CFB Particles, and 10 µl of DF Solution. Mix immediately by vortexing at maximum speed for 20 seconds.**

8. **Incubate sample tube on a tube rotator for 20 minutes at room temperature at 10 rpm.**
Adjust rotator angle to approximately 45 degrees for better mixing.

9. **Remove the tube from rotator and place on a compatible 15 ml magnetic separation device (CAT# MBMS-31550) to magnetize the MAG-CFB Particles for 20 minutes or until the magnetic particles are completely cleared from solution.**

10. **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.

Wash Steps

11. **Remove the sample tube off the magnetic separation device.**

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

13. **Transfer all of the solution from the 15 ml tube to a new 1.5 ml centrifuge tube.**

14. **Place the 1.5 ml sample tube on a compatible 1.5 ml magnetic separation device (CAT#MBMS-10) to magnetize the MAG-CFB Particles at room temperature for 5 minutes or until the magnetic particles are completely cleared from solution.**

15. **Remove and discard the cleared supernatant.** Do not disturb the attracted beads while aspirating the supernatant.

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

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

18. **Remove and discard the cleared supernatant.** Do not disturb the attracted beads while aspirating the supernatant.

19. **Remove the sample tube off the magnetic separation device.**

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

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

22. **Remove and discard the cleared supernatant.** Do not disturb the attracted beads while aspirating the supernatant.

23. **Repeat Steps 19-22 for a second CFW2 Buffer wash step.**

24. **With the sample tube on the magnetic separation device, air dry the MAG-CFB Particles for 10 minutes. Remove any residual liquid with a pipette.**
Note: It is critical to completely remove all liquid from the tube.

Elution Steps

25. **Remove the sample tube containing the MAG-CFB Particles off the magnetic separation device.**

26. **Add 50-100 µl Elution Buffer and completely resuspend the MAG-CFB Particles by vortexing at maximum speed for 10 seconds.**
Note: Heat Elution Buffer at 60°C to improve yield.

27. **Incubate at room temperature for 10 minutes.**

28. **Place the tubes back on the magnetic separation device and wait 5 minutes or until the magnetic particles are completely cleared from elution buffer.**

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

Isolation steps

Binding Steps

3. **Transfer 7 ml (plasma+SE Buffer) from the stabilized plasma sample to a new 50 ml conical tube.**

4. **Add 150 µl Pro K Solution, and mix well by vortexing at maximum speed for 20 seconds.**

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

6. **Add 7 ml CFL Buffer and mix well by vortexing at maximum speed for 60 seconds, and incubate sample at room temperature for 10 minutes.**

7. **Add 5.25 ml 100% ethanol and 20 µl MAG-CFB Particles, and 10 µl of DF Solution. Mix immediately by vortexing at maximum speed for 20 seconds.**
⚠Complete resuspension of the MAG-CFB Particles is crucial for obtaining purity.

8. **Incubate sample tube on a tube rotator for 20 minutes at room temperature at 10 rpm.**
Adjust rotator angle to approximately 45 degrees for better mixing.

9. **Remove the tube from rotator and place on a compatible 50 ml magnetic sepration device (CAT# MBMS-31550) to magnetize the MAG-CFB Particles for 30 minutes or until the magnetic particles are completely cleared from solution.**

10. **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.

Wash Steps

11. **Remove the sample tube off the magnetic separation device.**

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

13. **Transfer all of the solution from the 50 ml tube to a new 1.5 ml centrifuge tube.**

14. **Place the 1.5 ml sample tube on a compatible 1.5 ml magnetic separation device (CAT#MBMS-10) to magnetize the MAG-CFB Particles at room temperature for 5 minutes or until the magnetic particles are completely cleared from solution.**

15. **Remove and discard the cleared supernatant.** Do not disturb the attracted beads while aspirating the supernatant.

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

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

cfKapture™ 21 Kit : 5 ml plasma sample

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) from each product supplier.

- ☐ Nuclease-free 1.5 ml microcentrifuge tubes
- ☐ 15ml conical tubes
- ☐ 50ml conical tubes
- ☐ Magnetic separation device for 1.5 ml microcentrifuge tube (CAT# MBMS-10)
- ☐ Magnetic separation device for 50 ml conical tubes. (CAT# MBMS-31550)
- ☐ 100% Ethanol
- ☐ Vortex
- ☐ Tube rotator
- ☐ Water bath, incubator or heat block capable of 60°C

Things to do before starting

- ☐ Prepare CFW1 Buffer, CFW2 Buffer according to the "Preparation of Reagents" section on page 2
- ☐ Perheat and warm water bath, incubator or heat block to 60°C
- ☐ Warm Elution Buffer to 60°C

Preparing serum/plasma for stabilization

1. **Add 2ml of SE Buffer to a 15ml conical tube.**
   This tube will be used to stabilize the separated plasma from whole blood samples outlined below. 2mL of SE Buffer can stabilize up to 5 mL of plasma.

2. **Prepare serum/plasma from whole blood:**

⚠ **If processing 9-10ml whole blood from a Blood STASIS™ 21 tube (CAT# BS21-CF10-100), centrifugation steps can be performed at room temperature. If not processing from a Blood STASIS™ 21 tube, all centriguation steps need to be performed at 4°C.**

   a. Centrifuge whole blood samples at 2,000 g at room temperature.

   b. Transfer the plasma to a new 15 ml conical tube.

   c. Centrifuge the plasma sample again at 16,000 g for 10 min at room temperature to remove any residual blood and cell debris.

   d. Transfer 4-5 ml of clear plasma to the conical tube containing SE Buffer from step 1 to stabiize the plasma.

   e. Stabilized plasma can be stored at room temperature for 21 days or proceed to isolation process.

cfKapture™ 21 Kit : 4 ml plasma sample protocol

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) from each product supplier.

- ☐ Nuclease-free 1.5 ml microcentrifuge tubes
- ☐ 15 ml conical tubes
- ☐ Magnetic separation device for 1.5 ml microcentrifuge tube (CAT# MBMS-10)
- ☐ Magnetic separation device for 15 ml conical tubes. (CAT# MBMS-31550)
- ☐ 100% Ethanol
- ☐ Vortex
- ☐ Tube rotator
- ☐ Water bath, incubator or heat block capable of 60°C

Things to do before starting

- ☐ Prepare CFW1 Buffer, CFW2 Buffer according to the "Preparation of Reagents" section on page 2
- ☐ and Perheat and warm water bath, incubator or heat block to 60°C
- ☐ Warm Elution Buffer to 60°C

Preparing serum/plasma for stabilization

1. **Add 2ml of SE Buffer to a 15ml conical tube.**
   This tube will be used to stabilize the separated plasma from whole blood samples outlined below. 2mL of SE Buffer can stabilize up to 5 mL of plasma.

2. **Prepare serum/plasma from 9-10ml whole blood:**

⚠ **If processing 9-10ml whole blood from a Blood STASIS™ 21 tube (CAT# BS21-CF10-100), centrifugation steps can be performed at room temperature. If not processing from a Blood STASIS™ 21 tube, all centriguation steps need to be performed at 4°C.**

   a. Centrifuge whole blood samples at 2,000 g.

   b. Transfer the plasma to a new 15 ml conical tube.

   c. Centrifuge the plasma sample again at 16,000 g for 10 min to remove any residual blood and cell debris.

   d. Transfer 4-5 ml of clear plasma to the conical tube containing SE Buffer from step 1 to stabiize the plasma.

   e. Stabilized plasma can be stored at room temperature for 21 days or proceed to isolation process.

Isolation steps

Binding Steps

3. **Transfer 5.6 ml stabilized plasma prepared from step 2 to a new 50 ml conical tube.**
   Note: The 280 **µ**l sample volume factors in the dilution factor.

4. **Add 120 µl Pro K Solution, and mix well by vortexing at maximum speed for 20 seconds.**

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

6. **Add 5.6 ml CFL Buffer and mix well by vortexing at maximum speed for 60 seconds, and incubate sample at room temperature for 5 minutes.**

7. **Add 4.2 ml 100% ethanol and 20 µl MAG-CFB Particles, and 10 µl of DF Solution. Mix immediately by vortexing at maximum speed for 20 seconds.**
   ⚠ Complete resuspension of the MAG-CFB Particles is crucial for obtaining purity.

8. **Incubate sample tube on a tube rotator for 20 minutes at room temperature at 10 rpm.**
   Adjust rotator angle to approximately 45 degrees for better mixing.

9. **Remove the tube from rotator and place on a compatible 50 ml magnetic sepration device (CAT# MBMS-31550) to magnetize the MAG-CFB Particles for 30 minutes or until the magnetic particles are completely cleared from solution.**

10. **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.

Wash Steps

11. **Remove the sample tube off the magnetic separation device.**

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

13. **Transfer all of the solution from the 15 ml tube to a new 1.5 ml centrifuge tube.**

14. **Place the 1.5 ml sample tube on a compatible 1.5 ml magnetic separation device (CAT#MBMS-10) to magnetize the MAG-CFB Particles at room temperature for 5 minutes or until the magnetic particles are completely cleared from solution.**

15. **Remove and discard the cleared supernatant.** Do not disturb the attracted beads while aspirating the supernatant.

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

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

18. **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.

19. **Remove the sample tube off the magnetic separation device.**

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

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

22. **With the sample still on the magnetice separation device, remove and discard the cleared supernatant.** Do not disturb the attracted beads while aspirating the supernatant.

23. **Repeat Steps 19-22 for a second CFW2 Buffer wash step.**

24. **With the sample tube still on the magnetic separation device, air dry the MAG-CFB Particles for 10 minutes. Remove any residual liquid with a pipette.**
    Note: It is critical to completely remove all liquid from the tube.

Elution Steps

25. **Remove the sample tube containing the MAG-CFB Particles off the magnetic separation device.**

26. **Add 30-50 µl Elution Buffer and completely resuspend the MAG-CFB Particles by vortexing at maximum speed for 10 seconds.**
    Note: Heat Elution Buffer at 60°C to improve yield.

27. **Incubate at room temperature for 10 minutes.**

28. **Place the tubes back on the magnetic separation device and wait 5 minutes or until the magnetic particles are completely cleared from elution buffer.**

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