OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

Mag-Bind® cfDNA Kit

M3298-00	5 preps
M3298-01	50 preps
M3298-02	200 Preps

February 2017

For research use only.Not intended for diagnostic testing.

Mag-Bind® cfDNA Kit

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Manual Revision: February 2017

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Introduction

The Mag-Bind® cfDNA Kit is designed for rapid and reliable isolation of circulating DNA from 500-4000 µL plasma/serum samples. The Mag-Bind® cfDNA Kit can be processed manually with 15 mL centrifuge tubes or with automated platforms. The procedure eliminates the needs for funnels and vacuum steps providing hands-free operation in automated protocols. The uniquely formulated binding buffer from Omega Bio-tek allows for large samples volumes to be processed in automated formats with 4 mL serum or plasma being processed in a 24-well plate. The magnetic response time of the Mag-Bind® Particles CH allows for fast magnetization during steps requiring high volumes. The high binding capability decreases the amount of magnetic particles required thereby reducing the elution volume; up to 4 mL serum or plasma can be eluted in 50 µL.

This system combines the reversible nucleic acid-binding properties of Mag-Bind® paramagnetic particles with a unique binding system that targets smaller DNA fragments (150-400 bp) and minimizes binding of larger fragments such as gDNA.

If the desired target fragment is >300 bp, please consult with your Omega Bio-tek representative for a product that will fit your needs.

Utilizing paramagnetic particles provides high-quality DNA that is suitable for direct use in most downstream applications, such as qPCR and Next Generation Sequencing.

IMPORTANT: If automating this process on your liquid handler or magnetic processor please contact your Omega Bio-tek representative for instrument specific instructions.

Kit Contents

Product	M3298-00	M3298-01	M3298-02
Preps	5	50	200
Mag-Bind® Particles CH	200 µL	2.0 mL	7 mL
DS Buffer	1.5 mL	20 mL	80 mL
JSB Buffer	25 mL	225 mL	4 x 220 mL
VHB Buffer	5.5 mL	55 mL	2 x 110 mL
SPW Wash Buffer	2.5 mL	2 x 25 mL	3 x 50 mL
Elution Buffer	15 mL	150 mL	2 x 250 mL
Proteinase K Solution	350 µL	4 mL	14 mL
User Manual	✓	✓	✓

Storage and Stability

All of the Mag-Bind® cfDNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® Particles CH should be stored at 2-8°C for long-term use. Proteinase K Solution can be stored at room temperature for up to 12 months. For long-term storage, store Proteinase K Solution at 2-8°C.

Preparing Reagents

1. Prepare VHB Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M3298-00	7 mL
M3298-01	70 mL
M3298-02	140 mL per bottle

2. Dilute SPW Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M3298-00	10 mL
M3298-01	100 mL per bottle
M3298-02	350 mL 200 mL per bottle

3. Shake or vortex the Mag-Bind® Particles CH to fully resuspend the particles before use. The particles must be fully suspended during use to ensure proper binding.

Mag-Bind® cfDNA Kit - Protocol for 500-1000 µL Serum/Plasma

IMPORTANT: If automating this process on your liquid handler or magnetic processor, please contact your Omega Bio-tek representative for instrument specific instructions.

Materials and Reagents to be Supplied by User:

- 100% ethanol
- Magnetic separation device for 2 mL tubes or for 15 mL centrifuge tubes and 1.5/2.0 mL tubes (Omega Bio-tek Cat# MSD-02)
- Vortexer
- Incubator capable of 60°C
- 15 mL centrifuge tubes
- 1.5 mL microcentrifuge tubes

Before Starting:

- Prepare VHB Buffer and SPW Wash Buffer according to the "Preparing Reagents" section on Page 4
- Set Incubator to 60°C
- Shake or vortex the Mag-Bind® Particles CH to fully resuspend the particles before use

1. Add 500-1000 µL plasma/serum samples to a 15 mL centrifuge tube (not provided). Bring the volume up to 1 mL with Elution Buffer (provided with this kit) if the sample volume is less than 1 mL.

2. Add 15 µL Proteinase K Solution.

3. Add 67 µL DS Buffer.

4. Vortex at maximum speed or pipet up and down to mix thoroughly.

5. Incubate at 60°C for 20 minutes. Mix by inverting or shaking every 10 minutes.

6. Add 1 mL JSB Buffer. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.

7. Add 10 µL Mag-Bind® Particles CH. Invert the sample 10 times or pipet up and down to mix. Incubate for 10 minutes at room temperature with continuous mixing. The samples must be mixed throughout the 10 minute incubation period by shaking or rocking. ***Do not vortex at high speeds*** as this will cause excess foaming that can reduce yield. The speed of mixing should be set to continuously keep the Mag-Bind® Particles CH resuspended in solution.

8. Transfer 1 mL lysate to a 1.5 mL microcentrifuge tube (not provided).

9. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

10. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

11. Transfer the remaining lysate from Step 7 to the 1.5 mL microcentrifuge tube used in the previous steps.

12. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution

13. Remove the tube containing the Mag-Bind® Particles CH from the magnetic separation device.

14. Add 500 µL VHB Buffer.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

15. Resuspend the Mag-Bind® Particles CH by vortexing for 1 minute or pipetting up and down 20 times.

    Note: Complete resuspension of the Mag-Bind® Particles CH is critical for obtaining good purity.

16. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

17. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

18. Remove the tube containing the Mag-Bind® Particles CH from the magnetic separation device.

19. Add 500 µL VHB Buffer.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

20. Resuspend the Mag-Bind® Particles CH by vortexing for 1 minute or pipetting up and down 20 times.

    Note: Complete resuspension of the Mag-Bind® Particles CH is critical for obtaining good purity.

21. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

22. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

23. Remove the tube containing the Mag-Bind® Particles CH from the magnetic separation device.

24. Add 500 µL SPW Wash Buffer.

    Note: SPW Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

25. Resuspend the Mag-Bind® Particles CH by vortexing for 1 minute or pipetting up and down 20 times.

26. Let sit at room temperature for 1 minute.

27. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

28. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

29. Repeat Steps 23-28 for a second SPW Wash Buffer wash step.

30. Remove the tube from the magnetic separation device for approximately 30 seconds.

31. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH.

32. Aspirate and discard the residual SPW Wash Buffer.

33. Leave the tube on the magnetic separation device for 5 minutes to dry the Mag-Bind® Particles CH.

34. Remove the tube containing the Mag-Bind® Particles CH from the magnetic separation device.

35. Add 30-60 µL Elution Buffer. Resuspend the Mag-Bind® Particles CH by vortexing or pipetting up and down 20 times.

36. Let sit at room temperature for 5 minutes.

37. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

38. Transfer the cleared supernatant containing purified DNA to a clean microplate or 1.5 mL microcentrifuge tube (not provided).

39. Store DNA at -20°C.

Mag-Bind® cfDNA Kit - Protocol for 1001-2000 µL Serum/Plasma

IMPORTANT: If automating this process on your liquid handler or magnetic processor, please contact your Omega Bio-tek representative for instrument specific instructions.

Materials and Reagents to be Supplied by User:

- 100% ethanol
- Magnetic separation device for 24-well deep-well plates (AlpAqua Magnum FLX24) or for 15 mL centrifuge tubes and 1.5/2.0 mL tubes (Omega Bio-tek Cat# MSD-02)
- Vortexer
- Incubator capable of 60°C
- 1.5 mL microcentrifuge tubes
- 24-well deep-well plate or 15 mL centrifuge tube compatible with magnetic separation device used

Before Starting:

- Prepare VHB Buffer and SPW Wash Buffer according to the "Preparing Reagents" section on Page 4
- Set Incubator to 60°C
- Shake or vortex the Mag-Bind® Particles CH to fully resuspend the particles before use

1. Add 1000-2000 µL plasma/serum samples to a 15 mL centrfuge tube or 24-well deep-well plate (not provided). Choose the correct plasticware depending on the magnetic separation device being used. Bring the volume up to 2 mL with Elution Buffer (provided with this kit) if the sample volume is less than 2 mL.

2. Add 30 µL Proteinase K Solution.

3. Add 135 µL DS Buffer.

4. Vortex at maximum speed or pipet up and down to mix thoroughly.

5. Incubate at 60°C for 20 minutes. Mix by inverting or shaking every 10 minutes.

6. Add 2 mL JSB Buffer. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.

7. Add 20 µL Mag-Bind® Particles CH. Invert the sample 10 times or pipet up and down to mix. Incubate for 10 minutes at room temperature with continuous mixing. The samples must be mixed throughout the 10 minute incubation period by shaking or rocking. ***Do not vortex at high speeds*** as this will cause excess foaming that can reduce yield. The speed of mixing should be set to continuously keep the Mag-Bind® Particles CH resuspended in solution.

8. Place the tube/plate on a magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

9. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

10. Remove the tube/plate containing the Mag-Bind® Particles CH from the magnetic separation device.

11. Add 1 mL VHB Buffer.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

12. Resuspend the Mag-Bind® Particles CH by vortexing for 1 minute or pipetting up and down 20 times.

    Note: Complete resuspension of the Mag-Bind® Particles CH is critical for obtaining good purity.

13. Transfer the resuspended Mag-Bind® Particles CH to a new 1.5 mL microcentrifuge tube (not provided) if using a 15 mL centrifuge tube for Steps 1-12. Use a magnetic separation device designed for 1.5/2.0 mL tubes for the remaining procedure. If using a 24-well deep well plate for Steps 1-12, continue to use the 24-well deep-well plate and 24-well magnet.

14. Place the tube/plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

15. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

16. Remove the tube/plate containing the Mag-Bind® Particles CH from the magnetic separation device.

17. Add 1 mL VHB Buffer.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

18. Resuspend the Mag-Bind® Particles CH by vortexing for 1 minute or pipetting up and down 20 times.

    Note: Complete resuspension of the Mag-Bind® Particles CH is critical for obtaining good purity.

19. Place the tube/plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

20. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

21. Remove the tube/plate containing the Mag-Bind® Particles CH from the magnetic separation device.

22. Add 1 mL SPW Wash Buffer.

    Note: SPW Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

23. Resuspend the Mag-Bind® Particles CH by vortexing for 1 minute or pipetting up and down 20 times.

24. Let sit at room temperature for 1 minute.

25. Place the tube/plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

26. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

27. Repeat Steps 21-26 for an additional SPW Wash Buffer.

28. Remove the tube/plate from the magnetic separation device for approximately 30 seconds.

29. Place the tube/plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH.

30. Aspirate and discard the residual SPW Wash Buffer.

31. Leave the tube/plate on thet magnetic separation device for 5 minutes to dry the Mag-Bind® Particles CH.

32. Remove the tube/plate containing the Mag-Bind® Particles CH from the magnetic separation device.

33. Add 50-100 µL Elution Buffer. Resuspend the Mag-Bind® Particles CH by vortexing or pipetting up and down 20 times.

34. Let sit at room temperature for 5 minutes.

35. Place the tube/plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

36. Transfer the cleared supernatant containing purified DNA to a clean microplate or 1.5 mL microcentrifuge tube (not provided).

37. Store DNA at -20°C.

Mag-Bind® cfDNA Kit - Protocol for 2000-4000 µL Serum/Plasma

IMPORTANT: If automating this process on your liquid handler or magnetic processor please contact your Omega Bio-tek representative for instrument specific instructions.

Materials and Reagents to be Supplied by User:

- 100% ethanol
- Magnetic separation device for 24-well deep-well plates (AlpAqua Magnum FLX24) or for 15 mL centrifuge tubes and 1.5/2.0 mL tubes (Omega Bio-tek Cat# MSD-02)
- Vortexer
- Incubator capable of 60°C
- 1.5 mL microcentrifuge tubes
- 24-well deep-well plate or 15 mL centrifuge tube compatible with magnetic separation device used

Before Starting:

- Prepare VHB Buffer and SPW Wash Buffer according to the "Preparing Reagents" section on Page 4
- Set Incubator to 60°C
- Shake or vortex the Mag-Bind® Particles CH to fully resuspend the particles before use

1. Add 2000-4000 µL plasma/serum sample to a 15 mL centrfuge tube or 24-well deep-well plate (not provided). Choose the correct plasticware depending on the magnetic separation device being used. Bring the volume up to 4 mL with Elution Buffer (provided with this kit) if the volume of sample is less than 4 mL.

2. Add 60 µL Proteinase K Solution.

3. Add 270 µL DS Buffer.

4. Vortex at maximum speed or pipet up and down to mix thoroughly.

5. Incubate at 60°C for 20 minutes. Mix by inverting or shaking every 10 minutes.

6. Add 4 mL JSB Buffer. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.

7. Add 30 µL Mag-Bind® Particles CH. Invert the sample 10 times or pipet up and down to mix. Incubate for 10 minutes at room temperature with continuous mixing. The samples must be mixed throughout the 10 minute incubation period by shaking or rocking. ***Do not vortex at high speeds*** as this will cause excess foaming that can reduce yield. The speed of mixing should be set to continuously keep the Mag-Bind® Particles CH resuspended in solution.

8. Place the tube/plate on a magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

9. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

10. Remove the tube/plate containing the Mag-Bind® Particles CH from the magnetic separation device.

11. Add 1 mL VHB Buffer.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

12. Resuspend the Mag-Bind® Particles CH by vortexing for 1 minute or pipetting up and down 20 times.

    Note: Complete resuspension of the Mag-Bind® Particles CH is critical for obtaining good purity.

13. Transfer the resuspended Mag-Bind® Particles CH to a new 1.5 mL centrifuge tube (not provided) if using a 15 mL centrifuge tube for Steps 1-12. Use a magnetic separation device designed for 1.5/2.0 mL tubes for the remaining procedure. If using a 24-well deep-well plate for Steps 1-12, continue to use the 24-well deep-well plate and 24-well magnet.

14. Place the tube/plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

15. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

16. Remove the tube/plate containing the Mag-Bind® Particles CH from the magnetic separation device.

17. Add 1 mL VHB Buffer.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

18. Resuspend the Mag-Bind® Particles CH by vortexing for 1 minute or pipetting up and down 20 times.

    Note: Complete resuspension of the Mag-Bind® Particles CH is critical for obtaining good purity.

19. Place the tube/plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

20. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

21. Remove the tube/plate containing the Mag-Bind® Particles CH from the magnetic separation device.

22. Add 1 mL SPW Wash Buffer.

    Note: SPW Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

23. Resuspend the Mag-Bind® Particles CH by vortexing for 1 minute or pipetting up and down 20 times.

24. Let sit at room temperature for 1 minute.

25. Place the tube/plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

26. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

27. Repeat Steps 21-26 for an additional SPW Wash Buffer.

28. Remove the tube/plate from the magnetic separation device for approximately 30 seconds.

29. Place the tube/plate on the magnetic separation device to magnetize the Mag-Bind Particles CH.

30. Aspirate and discard the residual SPW Wash Buffer.

31. Leave the tube/plate on thet magnetic separation device for 5 minutes to dry the Mag-Bind® Particles CH.

32. Remove the tube/plate containing the Mag-Bind® Particles CH from the magnetic separation device.

33. Add 50-100 µL Elution Buffer. Resuspend the Mag-Bind® Particles CH by vortexing or pipetting up and down.

34. Let sit at room temperature for 5 minutes.

35. Place the tube/plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

36. Transfer the cleared supernatant containing purified DNA to a clean microplate or 1.5 mL centrifuge tube (not provided).

37. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **(800-832-8896)**.

Problem	Cause	Solution
Low DNA yield	Incomplete resuspension of Mag-Bind® Particles CH	Resuspend the Mag-Bind® Particles CH by vortexing vigorously before use
	Loss of Mag-Bind® Particles CH during operation	Avoid disturbing the Mag-Bind® Particles CH during aspiration
	DNA remains bound to Mag-Bind® Particles CH	• Increase elution volume and let sit at room temperature for 15 minutes • Pipet up and down 50 to 100 times.
	DNA washed off	Dilute SPW Wash Buffer by adding appropriate volume of 100% ethanol prior to use (see Page 4 for instructions).
	Ethanol is not added into VHB buffer	Make sure to add 100% ethanol to the VHB Buffer (see Page 4 for instructions).
	Ethanol carryover	Dry the Mag-Bind® Particles CH at 37°C for 5 minutes before elution.
High Molecular Weight Co-Purification	Two VHB Wash Steps must be performed	Perform 2 VHB Wash steps as instructed in the manual. Increase the volume of wash buffer if necessary.
Problems in downstream applications	Salt carryover	SPW Wash Buffer must be at room temperature.
	Ethanol carryover	Dry the Mag-Bind® Particles CH at 37°C for 5 minutes before elution.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Magnetic Separation Device for 1.5/2.0 mL tubes	MSD-02
Elution Buffer (EB Buffer), 500 mL	PD089