Mag-Bind® cfDNA Kit

Revision No: 1.0

omega BIO-TEK

Quick Guide

Please visit www.omegabiotek.com for a downloadable user manual containing additional protocols, troubleshooting tips, and ordering information.

Product	M3298-00	M3298-01	M3298-02
Purifications	5	50	200
DS Buffer	1.5 mL	20 mL	80 mL
JSB Buffer	25 mL	9 x 25 mL	4 x 220 mL
GT7 Buffer v1.1	11 mL	110 mL	2 x 220 mL
SPW Buffer	2.5 mL	25 mL	2 x 50 mL
Elution Buffer	30 mL	250 mL	2 x 250 mL
Proteinase K Solution	350 µL	4 mL	14 mL
Mag-Bind® Particles CH	110 µL	1.1 mL	4.4 mL

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

Kit includes enough reagents for the specified number or purifications plus an additional 10% overage to ensure there is sufficient volume. Please be aware that the actual number of purifications may be lower due to pre-aliquoting reagents, processing partial plates, and automation platform used etc. Additional reagents are available for purchase separately.

Supplied by user:

- Magnetic separation device for 24-well deep-well plates (Alpaqua Magnum FLX24), for 15 mL centrifuge tubes, or for 1.5/2.0 mL microcentrifuge tubes
- Incubator capable of 60°C
- Shaker or rocker
- Vortexer
- 24-well deep-well plates or 15 mL centrifuge tubes compatible with magnetic separation device used
- 1.5/2.0 mL microcentrifuge tubes compatible with magnetic separation device used
- 100% ethanol
- Optional: microplate for DNA storage

Before starting:

- Prepare SPW Buffer according to directions on the bottle
- Set incubator to 60°C
- Shake or vortex the Mag-Bind® Particles CH to fully resuspend the particles

Please refer to the Mag-Bind® cfDNA Kit manual for 2 mL serum/plasma extracton and recovery of smaller fragments (<150 bp).

cfDNA Extraction and Purification from up to 1 mL Serum/Plasma

LYSE

1. Add up to 1 mL serum/plasma sample to a 15 mL centrifuge tube (not provided). Bring volume up to 1 mL with Elution Buffer if sample volume is less than 1 mL.
2. Add 15 µL Proteinase K Solution and 67 µL DS Buffer sequentially. Vortex at maximum speed or pipet up and down to mix thoroughly.
3. Incubate at 60°C for 20 minutes. Mix by inverting or shaking every 10 minutes. Let sit at room temperature for 10 minutes.

BIND

4. Add 1 mL JSB Buffer and 5 µL Mag-Bind® Particles CH. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.
5. Invert the sample 10 times or pipet up and down to mix. Let sit 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.
6. Transfer 1 mL lysate to a 1.5 mL microcentrifuge tube (not provided). 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. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH. Remove the tube from the magnetic separation device.
7. Transfer the remaining lysate from Step 5 to the 1.5 mL microcentrifuge tube used in the previous step. 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. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH. Remove the tube from the magnetic separation device.

WASH

8. Add 500 µL GT7 Buffer v1.1. Vortex for 2 minutes to resuspend the Mag-Bind® Particles CH. Complete resuspension of the Mag-Bind® Particles CH is critical for obtaining good purity.
9. 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 have cleared from solution. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH. Remove the tube from the magnetic separation device.
10. Repeat Steps 8-9 for a second GT7 Buffer v1.1 step.
11. Add 500 µL SPW Buffer diluted with 100% ethanol (see bottle for instructions). Vortex for 2 minutes to resuspend the Mag-Bind® Particles CH. Complete resuspension of the Mag-Bind® Particles CH is critical for obtaining good purity.
12. 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 have cleared from solution. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.
13. Remove the tube from the magnetic separation device.

WASH

14. Repeat Steps 11-12 for a second SPW Buffer step.
15. Remove the tube from the magnetic separation device for approximately 30 seconds. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Aspirate and discard residual SPW Buffer.
16. Leave the tube on the magnetic separation device for 25 minutes to dry the Mag-Bind® Particles CH. Remove the tube containing the Mag-Bind® Particles CH from the magnetic separation device.

ELUTE

17. Add 30-60 µL Elution Buffer. Vortex at room temperature for 5 minutes to resuspend the Mag-Bind® Particles CH.
18. 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.
19. Transfer the cleared supernatant containing the purified DNA to a 1.5 mL micocentrifuge tube or clean microplate (not provided). Store DNA at -20°C.

cfDNA Extraction and Purification from up to 4 mL Serum/Plasma

LYSE

1. Add up to 4 mL serum/plasma sample to a 15 mL centrifuge tube (not provided) or 24-well deep-well plate (not provided). Choose the correct plasticware depending on the magnetic separation device being used. Bring volume up to 4 mL with Elution Buffer if sample volume is less than 4 mL.
2. Add 60 µL Proteinase K Solution and 270 µL DS Buffer sequentially. Vortex at maximum speed or pipet up and down to mix thoroughly.
3. Incubate at 60°C for 30 minutes. Mix by inverting or shaking every 10 minutes. Let sit at room temperature for 10 minutes.

BIND

4. Add 4 mL JSB Buffer and 20 µL Mag-Bind® Particles CH. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.
5. Invert the sample 10 times or pipet up and down to mix. Let sit 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.
6. Place the tube/plate on a magentic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are cleared completely from solution. Aspirate and discard the cleared supernatant. Do not disturb the particles. Remove the tube/plate from the magnetic separation device.

WASH

7. Add 1 mL GT7 Buffer v1.1. Vortex 5 minutes to resuspend the Mag-Bind® Particles CH. Complete resuspension of the Mag-Bind® Particles CH is critical for obtaining good purity.
8. 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 1-7. Use a magentic separation device designed for 1.5/2.0 mL tubes for the remaing procedure. If using a 24-well deep-well plate for Steps 1-7, continue to use the 24-well deep-well plate and a 24-well magnet.
9. 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 cleared from solution. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH. Remove the tube/plate from the magnetic separation device.
10. Add another 1 mL GT7 Buffer v1.1. Vortex 5 minutes to resuspend the Mag-Bind® Particles CH. Complete resuspension of the Mag-Bind® Particles CH is critical for obtaining good purity.
11. 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 cleared from solution. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH. Remove the tube/plate from the magnetic separation device.
12. Add 1 mL SPW Buffer diluted with 100% ethanol (see bottle for instructions). Vortex for 5 minutes to resuspend the Mag-Bind® Particles CH.
13. 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. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.
14. Remove the tube/plate from the magnetic separation device.
15. Repeat Steps 12-13 for a second SPW Buffer step.
16. Remove the tube/plate from the magnetic separation device for approximately 30 seconds. Place the tube/plate back on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Aspirate and discard the residual SPW Buffer.
17. Leave the tube/plate on the magnetic separation device for 25 minutes to dry the Mag-Bind® Particles CH. Remove the tube/plate containing the Mag-Bind® Particles CH from the magnetic separation device.

ELUTE

18. Add 50-100 µL Elution Buffer. Vortex at room temperature for 5 minutes to resuspend the Mag-Bind® Particles CH.
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. Transfer the cleared supernatant containing purified DNA to a 1.5 mL microcentrifuge tube or clean microplate (not provided). Store DNA at -20°C.