OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

Mag-Bind® Circulating DNA Kit

M3291-00	1 x 24 preps
M3291-01	4 x 24 preps

October 2014

For research use only.Not intended for diagnostic testing.

Mag-Bind® Circulating DNA Kit

Table of Contents

Manual Revision: October 2014

OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction

The Mag-Bind® Circulating DNA Kit is designed for rapid and reliable isolation of circulating DNA from 500-2000 µL plasma/serum samples. The Mag-Bind® Circulating DNA Kit can be processed manually with 15 mL centrifuge tubes or with automated platforms using 24-well plates. The procedure eliminates the needs for funnels and vacuum steps providing hands-free operation in automated protocols.

This system combines the reversible nucleic acid-binding properties of Mag-Bind® paramagnetic particles with a unique binding system that targets smaller DNA fragments(<300 bp) and minimizes the binding 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.

Overview

If using the Mag-Bind® Circulating DNA Kit for the first time, please read this booklet in its entirety to become familiar with the procedures. Blood cells are lysed in a specially formulated buffer. DNA is isolated from the lysates in one step by binding to Mag-Bind® Particles' surfaces. The paramagnetic particles are separated from the lysates by using a magnetic separation device. After a few rapid wash steps to remove trace contaminants, DNA is eluted in Elution Buffer.

Kit Contents

Product	M3291-00	M3291-01
Preps	1 x 24	4 x 24
Mag-Bind® Particles RQ	5.2 mL	20.8 mL
Carrier RNA Solution	260 µL	1.1 mL
DCL Buffer	45 mL	180 mL
ACB Buffer	40 mL	160 mL
VHB Buffer	44 mL	4 x 44 mL
DNA Wash Buffer	25 mL	4 x 25 mL
Proteinase K Solution	5.2 mL	22 mL
Elution Buffer	40 mL	150 mL
User Manual	✓	✓

Storage and Stability

All of the Mag-Bind® Circulating DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® Particles RQ 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. Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M3291-00	100 mL
M3291-01	100 mL per bottle

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

Kit	100% Ethanol to be Added
M3291-00	56 mL
M3291-01	56 mL per bottle

3. Prepare ACB Buffer with 100% isopropanol as follows and store at room temperature.

Kit	100% Isopropanol to be Added
M3291-00	60 mL
M3291-01	240 mL

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

Mag-Bind® Circulating DNA Kit - Protocol for 1-2 mL Serum/ Plasma

Materials and Reagents to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Magnetic separation device for 24-well plates (Cat# A000270) or Magnetic Separator for 15 mL centrifuge tubes
- Vortexer
- 24-well plate (10 mL) (Cat# Whatman 7701-5102) or 15 centrifuge tubes
- Incubator capable of 60°C
- 1.5 mL microcentrifuge tubes
- Optional: PBS or nuclease-free water

Before Starting:

- Prepare ACB Buffer, DNA Wash Buffer, and VHB Buffer according to the "Preparing Reagents" section on Page 4
- Set Incubator to 60°C

1. Add 1-2 mL plasma/serum samples to a 10 mL 24-well plate (not provided) or 15 mL centrifuge tube (not provided). Choose the correct plasticware depending on the magnetic stand being utilized to process the samples. Bring the volume up to 2 mL with Elution Buffer (provided with this kit) if the volume of sample is less than 2 mL.

2. Add 200 µL Proteinase K Solution to each sample.

3. Add 1.6 mL DCL Buffer to each sample.

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

5. Incubate at 60°C for 30 minutes. Mix by inverting or shaking every 10 minutes.

6. Add 3.6 mL ACB Buffer and 10 µL Carrier RNA Solution to each sample. Vortex at maximum speed for 30 seconds or pipet up and down to mix.

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

7. Add 200 µL Mag-Bind® Particles RQ . Vortex at maximum speed for 10 minutes or pipet up and down to mix. Continiously mix the samples throughout the 10 minute incubation period.

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

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

10. Remove the plate containing the Mag-Bind® Particles RQ from the magnetic separation device.

11. Add 3 mL VHB Buffer to each sample.

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

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

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

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

14. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.

15. Remove the plate containing the Mag-Bind® Particles RQ from the magnetic separation device.

16. Add 3 mL DNA Wash Buffer to each sample.

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

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

18. Let sit at room temperature for 1 minute.

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

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

21. Repeat Steps 15-20 using 800 µL DNA Wash Buffer.

22. Remove the plate from the magnetic separation device for approximately 30 seconds.

23. Place the plate on the magnetic separation device to magnetize the Mag-Bind Particles RQ.

24. Aspirate and discard the residual DNA Wash Buffer

25. Leave the plate on thet magnetic separation device

26. Add 500 µL water to each sample and immediately remove within 30 seconds.

27. Remove the plate containing the Mag-Bind® Particles RQ from the magnetic separation device.

28. Add 200-400 µL Elution Buffer or nuclease-free water to elute DNA from the Mag-Bind® Particles RQ. Resuspend the Mag-Bind® Particles RQ by pipetting up and down or vortexing.

29. Let sit at room temperature for 5 minutes.

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

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

32. Store DNA at -20°C.

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

Materials and Reagents to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Magnetic separation device for 24-well plates (Cat# A000270) or Magnetic Separator for 15 mL centrifuge tubes
- Vortexer
- 24-well plate (10 mL) (Cat# Whatman 7701-5102) or 15 centrifuge tubes
- Incubator capable of 60°C
- 1.5 mL microcentrifuge tubes
- Optional: PBS or nuclease-free water

Before Starting:

- Prepare ACB Buffer, DNA Wash Buffer, and VHB Buffer according to the "Preparing Reagents" section on Page 4
- Set Incubator to 60°C

1. Add 500-1000 µL plasma/serum samples to a 10 mL 24-well plate (not provided) or 15 mL centrifuge tube (not provided). Choose the correct plasticware depending on magnetic stand being utilized to process the samples. Bring the volume up to 1 mL with Elution Buffer (provided with this kit) if volume of sample is less than 1 mL.

2. Add 100 µL Proteinase K Solution to each sample.

3. Add 800 µL DCL Buffer to each sample.

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

5. Incubate at 60°C for 30 minutes. Mix by inverting or shaking every 10 minutes.

6. Add 1.8 mL ACB Buffer and 5 µL Carrier RNA Solution to each sample. Vortex at maximum speed for 30 seconds or pipet up and down to mix.

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

7. Add 100 µL Mag-Bind® Particles RQ . Vortex at maximum speed for 10 minutes or pipet up and down to mix. Continiously mix the samples throughout the 10 minute incubation period.

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

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

10. Remove the plate containing the Mag-Bind® Particles RQ from the magnetic separation device.

11. Add 2 mL VHB Buffer to each sample.

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

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

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

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

14. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.

15. Remove the plate containing the Mag-Bind® Particles RQ from the magnetic separation device.

16. Add 2 mL DNA Wash Buffer to each sample.

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

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

18. Let sit at room temperature for 1 minute.

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

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

21. Repeat Steps 15-20 using 800 µL DNA Wash Buffer.

22. Remove the plate from the magnetic separation device for approximately 30 seconds.

23. Place the plate on the magnetic separation device to magnetize the Mag-Bind Particles RQ.

24. Aspirate and discard the residual DNA Wash Buffer

25. Leave the plate on thet magnetic separation device

26. Add 500 µL water to each sample and immediately remove within 30 seconds.

27. Remove the plate containing the Mag-Bind® Particles RQ from the magnetic separation device.

28. Add 100-200 µL Elution Buffer or nuclease-free water to elute DNA from the Mag-Bind® Particles RQ. Resuspend the Mag-Bind® Particles RQ by pipetting up and down or vortexing.

29. Let sit at room temperature for 5 minutes.

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

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

32. 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 RQ	Resuspend the Mag-Bind® Particles RQ by vortexing vigorously before use
	Loss of Mag-Bind® Particles RQ during operation	Avoid disturbing the Mag-Bind® Particles RQ during aspiration
	DNA remains bound to Mag-Bind® Particles RQ	Increase elution volume and let sit at for 15 minutes; pipet up and down 50 to 100 times
	DNA washed off	Dilute DNA Wash Buffer by adding appropriate volume of ethanol prior to use (see Page 4 for instructions)
	Ethanol is not added into VHB buffer	Make sure to add ethanol to the VHB Buffer (see Page 4 for instructions)
	Ethanol carryover	Dry the Mag-Bind® Particles RQ at 37°C for 5 minutes before elution
Mag-Bind® Particles RQ do not completely clear from solution	Too short of magnetizing time	Increase collection time on the magnet
Problems in downstream applications	Salt carryover	DNA Wash Buffer must be at room temperature
	Ethanol carryover	Dry the Mag-Bind® Particles RQ 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 Stand for 1.5/2.0 mL tubes	MSD-02
Elution Buffer (EB Buffer), 500 mL	PD089
RNase A, 400 µL	AC117
RNase A, 5 mL	AC118
24-well Magnetic Stand	A000270

HiBind®, E.Z.N.A.®, and MicroElute® are registered trademarks of Omega Bio-tek, Inc.
PCR is a patented process of Hoffman-La Roche. Use of the PCR process requires a license.

Notes:

Notes: