Mag-Bind® Blood DNA 96 Kit

Table of Contents

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OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction

The Mag-Bind® Blood DNA 96 Kit is designed for rapid and reliable isolation of high-quality genomic DNA from 1-250 µL blood samples. This system combines the reversible nucleic acid-binding properties of Mag-Bind® paramagnetic particles with the time-proven efficiency of Omega Bio-tek's blood DNA isolation system to provide a fast and convenient method to isolated DNA from fresh or frozen blood. Utilizing paramagnetic particles provides high-quality DNA that is suitable for direct use in most downstream applications, such as amplification and enzymatic reactions.

Overview

If using the Mag-Bind® Blood DNA 96 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.

New in this Edition: This manual has been edited for content and redesigned to enhance user readability.

- Proteinase K is now supplied in a liquid form eliminating the resuspension step to prior to use.
- Proteinase K Solution can also be stored at room temperature for 12 months.
- Proteinase Storage Buffer is no longer included in the kit.

Kit Contents

Product	M6211-00	M6211-01	M6211-02
Preps	1 x 96	4 x 96	20 x 96
Mag-Bind® Particles C	1.1 mL	4 mL	20 mL
MSL Buffer	30 mL	120 mL	600 mL
MP Buffer	20 mL	80 mL	400 mL
SPM Wash Buffer	30 mL	120 mL	2 x 300 mL
RNase A	500 µL	2 mL	10 mL
Proteinase K Solution	2.5 mL	9 mL	45 mL
Elution Buffer	20 mL	80 mL	400 mL
User Manual	✓	✓	✓

Storage and Stability

All components of the Mag-Bind® Blood DNA 96 Kit are guaranteed for at least 12 months from the date of purchase when stored as follows. Proteinase K Solution can be stored at room temperature for 12 months. For long-term storage (>12 months), store at 2-8°C. Store Mag-Bind® Particles C at 2-8°C. Store RNase A at -20°C. Store all other components at room temperature. Check buffers for precipitates before use. Redissolve any precipitates by warming to 37˚C.

Preparing Reagents

- Dilute SPM Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M6211-00	70 mL
M6211-01	280 mL
M6211-02	700 mL to each bottle

- Dilute MP Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M6211-00	30 mL
M6211-01	120 mL
M6211-02	600 mL

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

Mag-Bind® Blood DNA 96 Protocol (1-100 µL Blood)

The procedure below has been optimized for use with 1 to 100 µL FRESH or FROZEN blood samples. Buffy coat can also be used.

Materials and Equipment to be Supplied by User:

- Water bath, incubator, or heat block capable of 65°C
- 100% ethanol
- Magnetic separation device for 96-well microplates (Cat# MSD-01B)
- 96-well microplate (500 µL) (Cat# EZ9604)
- 96-well Round-well Plate (1.2 mL) (Cat# SSI1780)
- Sealing film (Cat# AC1200)
- Optional: PBS or nuclease-free water

Before Starting:

- Heat water bath, incubator, or heating block to 65°C
- Prepare SPM Wash Buffer and MP Buffer according to the Preparing Reagents section on Page 4

1. Add blood samples to a 96-well Round-well Plate (1.2 mL). Bring volume up to 200 µL with PBS (not provided) or Elution Buffer (provided with this kit).

2. Add 20 µL Proteinase K Solution to each sample. Mix by pipetting up and down 20 times.

3. Add 5 µL RNase A to each sample. Mix by pipetting up and down 20 times.

4. Add 200 µL MSL Buffer to each sample. Mix by pipetting up and down 20 times.

5. Incubate samples at 65°C for 20 minutes. Mix the samples once during incubation by pipetting up and down 5 times.

6. Cool samples to room temperature by sitting the plate at room temperature for 5 minutes.

7. Add 300 µL ethanol and 10 µL Mag-Bind® Particles C to each sample. Mix the samples by pipetting up and down 20 times.

8. Let sit at room temperature for 5 minutes. Mix the samples once during incubation by pipetting up and down 5 times.

9. Transfer 360 µL sample to a 96-well microplate (500 µL).

10. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles C. Let sit for 5-10 minutes.

    Note: If MSD-01B is used, the Mag-Bind® Particles C should collect at the corner of each well adjacent to the magnet.

11. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles C.

12. Remove the plate containing the Mag-Bind® Particles C from the magnetic separation device.

13. Repeat Steps 9-12 until remaining samples are completely transferred to the 96-well microplate. Remove any remaining droplets of liquid from the walls of the each well with a pipettor.

14. Add 400 µL MP Buffer to each sample.

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

15. Resuspend the Mag-Bind® Particles C by pipetting up and down 20 times.

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

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

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

18. Remove the plate containing the Mag-Bind® Particles C from the magnetic separation device.

19. Add 400 µL SPM Wash Buffer to each sample.

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

20. Resuspend the Mag-Bind® Particles C by pipetting up and down 20 times.

21. Let sit at room temperature for 1 minute.

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

23. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles C.

24. Repeat Steps 18-23 for a second SPM Wash Buffer wash step.

Optional: Remove the plate containing the Mag-Bind® Particles C from the magnetic separation device. Add 400 µL ethanol to each sample and resuspend the Mag-Bind® Particles C by pipetting up and down 20 times. Magnetize the Mag-Bind® Particles C and aspirate the supernatant once the Mag-Bind® Particles C have completely cleared from solution.

25. Leave the plate on the magnetic separation device for 5-10 minutes to air dry the Mag-Bind® Particles C. Remove any residue liquid with a pipettor.

    Note: Heating at 37°C is permitted to dry the Mag-Bind® Particles C faster.

26. Remove the plate containing the Mag-Bind® Particles C from the magnetic separation device.

27. Add 100-200 µL Elution Buffer or nuclease-free water to elute DNA from the Mag-Bind® Particles C. Resuspend the Mag-Bind® Particles by pipetting up and down 50 times.

28. Incubate at 65°C for 5 minutes.

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

30. Transfer the cleared supernatant containing purified DNA to a clean microplate (not supplied). Store the DNA at -20°C.

Mag-Bind® Blood DNA 96 Protocol (100-250 µL Blood)

The procedure below has been optimized for use with 100-250 µL FRESH or FROZEN blood samples. Buffy coat can also be used.

Materials and Equipment to be Supplied by User:

- Water bath, incubator, or heat block capable of 65°C
- 100% ethanol
- Magnetic separation device for 96-well microplates (Cat# MSD-01B)
- 96-well microplate (500 µL) (Cat# EZ9604)
- 96-well Round-well Plate (1.2 mL) (Cat# SSI1780)
- Sealing film (Cat# AC1200)
- Optional: PBS or nuclease-free water

Before Starting:

- Heat water bath, incubator, or heat block to 65°C
- Prepare SPM Wash Buffer and MP Buffer according to the Preparing Reagents section on Page 4

1. Add blood samples to a 96-well Round-well Plate (1.2 mL). Bring the volume up to 300 µL with PBS (not provided) or Elution Buffer (provided with this kit).

2. Add 20 µL Proteinase K Solution to each sample. Mix by pipetting up and down 20 times.

3. Add 5 µL RNase A to each sample. Mix by pipetting up and down 20 times.

4. Add 300 µL MSL Buffer to each sample. Mix by pipetting up and down 20 times.

5. Incubate sample at 65°C for 20 minutes. Mix the samples once during incubation by pipetting up and down 5 times.

6. Cool the sample to room temperature by sitting the plate at room temperature for 5 minutes.

7. Add 430 µL ethanol and 10 µL Mag-Bind® Particles C to each sample. Mix by pipetting up and down 20 times.

8. Let sit at room temperature for 5 minutes. Mix the samples once during incubation by pipetting up and down 5 times.

9. Transfer 360 µL of each sample to a 96-well microplate (500 µL).

10. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles C. Let sit for 5-10 minutes.

    Note: If MSD-01B is used, the Mag-Bind® Particles C should collect at the corner of each well adjacent to the magnet.

11. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles C.

12. Remove the plate containing the Mag-Bind® Particles C from the magnetic separation device.

13. Repeat Steps 9-12 until remaining samples are completely transferred into the round-bottom plate. Remove any remaining droplets of liquid from the walls of the each well with a pipettor.

14. Add 400 µL MP Buffer to each sample.

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

15. Resuspend the Mag-Bind® Particles C by pipetting up and down 20 times.

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

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

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

18. Remove the plate containing the Mag-Bind® Particles C from the magnetic separation device.

19. Add 400 µL SPM Wash Buffer to each sample.

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

20. Resuspend the Mag-Bind® Particles C by pipetting up and down 20 times.

21. Let sit at room temperature for 1 minute.

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

23. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles C.

24. Repeat Steps 18-23 for a second SPM Wash Buffer wash step.

Optional: Remove the plate containing the Mag-Bind® Particles C from the magnetic separation device. Add 400 µL ethanol to each sample and resuspend the Mag-Bind® Particles C by pipetting up and down 20 times. Magnetize the Mag-Bind® Particles C and aspirate the supernatant once the Mag-Bind® Particles C have completely cleared from solution.

25. Leave the plate on the magnetic separation device for 5-10 minutes to air dry the Mag-Bind® Particles C. Remove any residue liquid with a pipettor.

    Note: Heating at 37°C is permitted to dry the Mag-Bind® Particles C faster.

26. Remove the plate containing the Mag-Bind® Particles C from the magnetic separation device.

27. Add 100-200 µL Elution Buffer or nuclease-free water to elute DNA from the Mag-Bind® Particles C. Resuspend the Mag-Bind® Particles C by pipetting up and down 50 times.

28. Incubate at 65°C for 5 minutes.

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

30. Transfer the cleared supernatant containing purified DNA to a clean 96-well microplate (not supplied). Store the DNA at -20°C.

Mag-Bind® Blood DNA 96 Protocol (Dried Blood, Body Fluids, Sperms Spots)

Dried blood, body fluids, and sperm samples on filter paper can be processed using the following method. **Please note that this protocol needs TL Buffer and is not supplied with this kit)**. TL Buffer can be purchased separately from Omega Bio-tek, Inc. or its distributors.

Materials and Equipment to be Supplied by User:

- Water baths, incubators, or heat blocks capable of 65°C
- TL Buffer (Cat# PD061)
- 100% ethanol
- Microcentrifuge capable of at least 10,000 x *g*
- Magnetic separation device for 96-well microplates (Cat# MSD-01)
- 1.5 or 2 mL nuclease-free microcentrifuge tubes (Cat# SSI-1210-00 or SSI-1310-00)
- 96-well microplate (500 µL) (Cat# EZ9604)
- 96-well Round-well Plate (1.2 mL) (Cat# SSI1780)
- Sealing film (Cat# AC1200)
- Optional: PBS or nuclease-free water

Before Starting:

- Heat water bath, incubator, or heat block to 65°C
- Prepare SPM Wash Buffer and MP Buffer according to the Preparing Reagents section on Page 4

1. Cut or punch out the blood (or other sample) spot from the filter paper. Tear or cut filter into small pieces and place into a 1.5 or 2.0 mL microcentrifuge tube (not provided).

   Note: Use 1-4 punched circles (3 mm diameter) for each DNA isolation.

2. Add 200 µL TL Buffer (not provided) to each sample.

3. Add 20 µL Proteinase K Solution to each sample. Mix by pipetting up and down 20 times.

4. Add 5 µL RNase A to each sample. Mix by pipetting up and down 20 times.

5. Incubate at 55°C for 45-60 minutes. Vortex several times during incubation.

6. Briefly centrifuge the tube to collect any liquid droplets from inside the lid.

7. Add 210 µL MSL Buffer to each sample. Vortex for 20 seconds at maximum speed.

8. Incubate at 65°C for 15 minutes. Vortex several times during incubation.

9. Centrifuge at 10,000 x *g* for 10 minutes.

   Note: For maximum yield, collect any remaining liquid from the paper and transfer entire sample, including paper, to a Homogenizer Column (not supplied) and centrifuge at 10,000 x *g* for 2 minutes to collect all the lysate. Homogenizer Columns (Cat# HCR-001 and HCR-003) can be purchased separately from Omega Bio-tek, Inc.

10. Transfer the cleared samples into a 96-well Round-well Plate (1.2 mL).

11. Cool the sample to room temperature by sitting the plate at room temperature for 5 minutes.

12. Add 280 µL ethanol and 10 µL Mag-Bind® Particles C to each sample. Mix by pipetting up and down 20 times.

13. Let sit at room temperature for 5 minutes. Mix the samples once during incubation by pipetting up and down 5 times.

14. Transfer 360 µL of each sample to a 96-well microplate.

15. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles C. Let sit for 5-10 minutes.

    Note: If MSD-01B is used, the Mag-Bind® Particles C should collect at the corner of each well adjacent to the magnet.

16. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles C.

17. Remove the plate containing the Mag-Bind® Particles C from the magnetic separation device.

18. Repeat Steps 14-17 until remaining sample are completely transferred into the round-bottom plate. Remove any remaining droplets of liquid from the walls of each well with a pipettor.

19. Add 400 µL MP Buffer to each sample.

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

20. Resuspend the Mag-Bind® Particles C by pipetting up and down 20 times.

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

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

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

23. Remove the plate containing the Mag-Bind® Particles C from the magnetic separation device.

24. Add 400 µL SPM Wash Buffer to each sample.

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

25. Resuspend the Mag-Bind® Particles C by pipetting up and down 20 times.

26. Let sit at room temperature for 1 minute.

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

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

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

Optional: Remove the plate containing the Mag-Bind® Particles C from the magnetic separation device. Add 400 µL ethanol to each sample and resuspend the Mag-Bind® Particles C by pipetting up and down 20 times. Magnetize the Mag-Bind® Particles C and aspirate the supernatant once the Mag-Bind® Particles C have completely cleared from solution.

30. Leave the plate on the magnetic separation device for 5-10 minutes to air dry the magnetic particles. Remove any residue liquid with a pipettor.

    Note: Heating at 37°C is permitted to dry the Mag-Bind® Particles C faster.

31. Remove the plate containing the Mag-Bind® Particles C from the magnetic separation device.

32. Add 100-200 µL Elution Buffer or nuclease-free water to elute DNA from the Mag-Bind® Particles C. Resuspend the Mag-Bind® Particles C by pipetting up and down 50 times.

33. Incubate at 65°C for 5 minutes.

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

35. Transfer the cleared supernatant containing purified DNA to a clean 96-well microplate (not supplied). Store the 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 C	Resuspend the Mag-Bind® Particles C by vortexing vigorously before use.
	Frozen blood samples not mixed properly after thawing	Thaw the frozen blood at room temperature and gently mix the blood by inverting.
	Loss of Mag-Bind® Particles C during operation	Avoid disturbing the Mag-Bind® Particles C during aspiration.
	DNA remains bound to Mag-Bind® Particles C	Increase elution volume and incubate at 65°C for 5 minutes; pipet up and down 50 to 100 times.
	DNA washed off	Dilute SPM Wash Buffer by adding appropriate volume of ethanol prior to use (see Page 4 for instructions).
	Ethanol is not added into MP buffer	Make sure to add ethanol in the MP Buffer (see Page 4 for instructions).
Mag-Bind® Particles C do not completely clear from solution	Too short of magnetizing time	Increase collection time on the magnet.
Gel-like material in the eluted DNA	Blood is too old	Remove the gel-like material by centrifugation; recommend using fresh blood.
		Use 8 mM NaOH as elution buffer.
Problems in downstream applications	Salt carry-over	SPM Wash Buffer must be at room temperature.
	Ethanol carry-over	Dry the Mag-Bind® Particles C before elution.

Ordering Information

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

Product	Part Number
TL Buffer, 100 mL	PD061
MSL Buffer, 100 mL	PD070
Elution Buffer (EB Buffer), 100 mL	PDR048
Elution Buffer (EB Buffer), 500 mL	PD089
RNase A, 400 µL	AC117
RNase A, 5 mL	AC118
Omega Homogenizer Columns (50)	HCR001
Omega Homogenizer Columns (200)	HCR003
1.5 mL DNase/RNase-free Microcentrifuge Tubes	SSI-1210-00
2 mL DNase/RNase-free Microcentrifuge Tubes	SSI-1310-00
Magnetic Separation Device for Microplates	MSD-01
96-well Round-well Plates (1.2 mL)	SSI1780
96-well Microplates (500 µL)	EZ9604
Sealing Film	AC1200

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