OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

Mag-Bind® Blood DNA HDQ 96 Kit

M6399-00	1 x 96 preps
M6399-01	4 x 96 preps
M6399-02	20 x 96 preps

August 2013

For research use only.Not intended for diagnostic testing.

Mag-Bind® Blood DNA HDQ 96 Kit

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Introduction

The Mag-Bind® Blood DNA HDQ 96 Kit is designed for rapid and reliable isolation of high-quality genomic DNA from 100-200 µL blood samples. All heating steps that limit robotic applications have been removed to allow for faster processing. Mag-Bind® Particles HDQ provide quick magnetic response time reducing overall processing time. 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 HDQ 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.

Kit Contents

Product	M6399-00	M6399-01	M6399-02
Preps	1 x 96	4 x 96	20 x 96
Mag-Bind® Particles HDQ	2.2 mL	8 mL	40 mL
AL Buffer	30 mL	120 mL	600 mL
HDQ Binding Buffer	10 mL	40 mL	200 mL
VHB Buffer	55 mL	220 mL	2 x 440 mL
SPM Wash Buffer	30 mL	120 mL	2 x 300 mL
Proteinase K Solution (10 mg/mL)	1.1 mL	4.4 mL	22 mL
Elution Buffer	20 mL	80 mL	400 mL
User Manual	✓	✓	✓

Storage and Stability

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

Kit	100% Ethanol to be Added
M6399-00	70 mL
M6399-01	280 mL
M6399-02	700 mL per bottle

2. Prepare VHB Buffer as follows and store at room temperature.

Kit	100% Ethanol to be Added
M6399-00	70 mL
M6399-01	280 mL
M6399-02	560 mL

3. Prepare HDQ Binding Buffer as follows and store at room temperature.

Kit	100% Isopropanol to be Added
M6399-00	40 mL
M6399-01	160 mL
M6399-02	800 mL

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

Mag-Bind® Blood DNA HDQ 96 Protocol (100-200 µL Blood)

The procedure below has been optimized for use with 100-200 µL FRESH or FROZEN blood samples. Buffy coat can also be used.

Materials and Reagents to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Magnetic separation device for microplates (Cat# MSD-01B)
- Vortexer
- 96-well Microplate (500 µL) (Cat# EZ9604) or desired elution plate
- 96-well Round-well Plate (1.2 mL) (Cat# SSI1780)
- Sealing film (Cat# AC1200)
- Optional: PBS or nuclease-free water
- Optional: RNase A (10 mg/mL)

Before Starting:

- Prepare SPM Wash Buffer, HDQ Binding Buffer, and VHB 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 200 µL with PBS (not provided) or Elution Buffer (provided with this kit) if volume of blood is less than 200 µL.

2. Add 10 µL Proteinase K Solution to each sample. Vortex or pipet up and down 20 times to mix.

Optional: Add 5 µL RNase A to each sample. Vortex or pipet up and down 20 times to mix.

3. Add 230 µL AL Buffer to each sample. Vortex at maximum speed for 10 minutes.

4. Add 320 µL HDQ Binding Buffer and 20 µL Mag-Bind® Particles HDQ to each sample. Vortex at maximum speed for 10 minutes.

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

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

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

7. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

8. Add 600 µL VHB Buffer to each sample.

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

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

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

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

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

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

13. Repeat Steps 8-12 for a second VHB Buffer wash step.

14. Add 600 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.

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

16. Let sit at room temperature for 1 minute.

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

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

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

20. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

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

22. Let sit at room temperature for 10 minutes.

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

24. Transfer the cleared supernatant containing purified DNA to a clean 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 HDQ	Resuspend the Mag-Bind® Particles HDQ 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 HDQ during operation	Avoid disturbing the Mag-Bind® Particles HDQ during aspiration
	DNA remains bound to Mag-Bind® Particles HDQ	Increase elution volume and incubate at for 15 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 VHB buffer	Make sure to add ethanol to the VHB Buffer (see Page 4 for instructions)
Mag-Bind® Particles HDQ 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 HDQ before elution

Ordering Information

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

Product	Part Number
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

Notes: