Mag-Bind® Viral DNA/RNA 96 Kit

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OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction

The Mag-Bind® Viral DNA/RNA Kit is designed for rapid and reliable isolation of total nucleic acid from whole blood, serum, plasma, saliva, and other body fluids. The Mag-Bind® paramagnetic bead technology provides high-quality RNA or DNA, which is suitable for direct use in most downstream applications such as amplifications and enzymatic reactions. This system can be easily adapted to automated systems or centrifugation systems. The procedure can be scaled up or down, allowing purification from various amounts of starting material.

Overview

If using the Mag-Bind® Viral DNA/RNA Kit for the first time, please read this booklet to become familiar with the procedure and its various modifications. Samples are lysed in a specially formulated buffer containing detergent. Nucleic acid is bound to the surface of Mag-Bind® magnetic particles under proper condition. Proteins and cellular debris are efficiently washed with few wash steps. Pure RNA and DNA is then eluted in nuclease-free water or low ionic strength buffer. Purified RNA or DNA can be directly used in downstream applications without the need for further purification.

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 step to respuspend prior to use.
- Proteinase K Solution can be stored at room temperature for 12 months.
- Proteinase Storage Buffer is no longer included in the kit.

Kit Contents

Product	M6246-01	M6246-02	M6246-03
Preparations	1 x 96 preps	4 x 96 preps	12 x 96 preps
Mag-Bind® Particles CNR	1.1 mL	4.4 mL	13 mL
TNA Lysis Buffer	30 mL	110 mL	2 x 160 mL
VHB Buffer	22 mL	88 mL	3 x 88mL
Carrier RNA	1 mg	4 x 1 mg	12 x 1 mg
Proteinase K Solution	2.2 mL	8.8 mL	28 mL
SPR Wash Buffer	25 mL	2 x 50 mL	6 x 50 mL
Nuclease-free Water	35 mL	150 mL	2 x 200 mL
User Manual	✓	✓	✓

Storage and Stability

All of the Mag-Bind® Viral DNA/RNA Kit components are guaranteed for at least 24 months from the date of purchase when stored as follows. Mag-Bind® Particles CNR must be stored at 2-8°C. 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. Carrier RNA should be stored at -20°C after resuspension. All remaining components should be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form inTNA Lysis Buffer. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Preparing Reagents

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

Kit	100% Ethanol to be Added
M6246-01	28 mL
M6246-02	112 mL
M6246-03	112 mL per bottle

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

Kit	100% Ethanol to be Added
M6246-01	100 mL
M6246-02	200 mL per bottle
M6246-03	200 mL per bottle

- Add Nuclease-free Water to the tube containing lyophilized Carrier RNA to obtain a solution of 1 µg/µL. Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at –20°C. Do not freeze–thaw the aliquots of Carrier RNA more than 3 times.

Mag-Bind® Viral DNA/RNA Kit Protocol - 50 µL Sample Volume

Materials and Equipment to be Supplied by User:

- 100% Ethanol
- Isopropanol
- Magnetic Separation Device for 96-well plates (Cat# MSD-01)
- 96-well microplates (U or V bottom)

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4

1. Freshly prepare the following lysis mastermix per sample.

Buffer	Volume
TNA Lysis Buffer	60 µL
Carrier RNA	2 µL
Isopropanol	70 µL

2. Transfer 132 µL lysis mastermix to each well of a 96-well microplate.

3. Add 50 µL plasma or serum into each well. Mix by shaking for 1 minute. If using frozen samples, thaw at room temperature and mix well by shaking or pipetting up and down before proceeding to Step 4.

   Note: If the sample is less than 50 µL, bring the volume up to 50 µL with Nuclease-free water.

4. Add 5 µL Mag-Bind® Particles CNR and 10 µL Proteinase K Solution to each well. Mix by shaking for 5 minutes.

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

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

7. Remove the plate from the magnetic separation device.

8. Add 200 µL VHB Buffer to each well.

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

9. Resuspend the Mag-Bind® Particles CNR by shaking for 1 minute.

   Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles.

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

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

12. Remove the plate from the magnetic separation device.

13. Add 200 µL SPR Wash Buffer to each well.

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

14. Resuspend the Mag-Bind® Particles CNR by shaking for 1 minute.

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

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

17. Repeat Steps 12-16 for a second SPR Wash Buffer wash step.

18. Leave the plate on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles CNR. Remove any residual liquid with a pipettor.

19. Remove the plate from the magnetic separation device.

20. Add 20-50 µL Nuclease-free Water to each well.

    Note: Elution volume depends on plasticware and magnetic separation device used. The Mag-Bind® Particles CNR must be able to completely covered by the Nuclease-free Water.

21. Resuspend the Mag-Bind® Particles CNR by shaking for 2 minutes.

22. Let sit at room temperature for 10 minutes.

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

24. Transfer the cleared supernatant containing purified DNA/RNA to a clean plate. Store at -70°C.

Mag-Bind® Viral DNA/RNA Kit Protocol - 200 µL Sample Volume

Materials and Equipment to be Supplied by User:

- 100% Ethanol
- Isopropanol
- Magnetic Separation Device for 96-well plates (Cat# MSD-01)
- 96-well microplates (U or V bottom)

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4

1. Freshly prepare the following lysis mastermix per sample.

Buffer	Volume
TNA Lysis Buffer	240 µL
Carrier RNA	8 µL
Isopropanol	280 µL

2. Transfer 528 µL lysis mastermix to each well of a 96-well microplate.

3. Add 200 µL plasma or serum into each well. Mix by shaking for 1 minute. If using frozen samples, thaw at room temperature and mix well by shaking or pipetting up and down before proceeding to Step 4.

   Note: If the sample is less than 200 µL, bring the volume up to 200 µL with Nuclease-free water.

4. Add 10 µL Mag-Bind® Particles CNR and 20 µL Proteinase K Solution to each well. Mix by shaking for 5 minutes.

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

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

7. Remove the plate from the magnetic separation device.

8. Add 400 µL VHB Buffer to each well.

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

9. Resuspend the Mag-Bind® Particles CNR by shaking for 1 minute.

   Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles.

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

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

12. Remove the plate from the magnetic separation device.

13. Add 500 µL SPR Wash Buffer to each well.

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

14. Resuspend the Mag-Bind® Particles CNR by shaking for 1 minute.

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

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

17. Repeat Steps 12-16 for a second SPR Wash Buffer wash step.

18. Leave the plate on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles CNR. Remove any residual liquid with a pipettor.

19. Remove the plate from the magnetic separation device.

20. Add 50-100 µL Nuclease-free Water to each well.

    Note: Elution volume depends on plastic ware and magnetic separation device used. The Mag-Bind® Particles CNR must be able to completely covered by the Nuclease-free Water.

21. Resuspend the Mag-Bind® Particles CNR by shaking for 2 minutes.

22. Let sit at room temperature for 10 minutes.

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

24. Transfer the cleared supernatant containing purified DNA/RNA to a clean plate. Store at -70°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 **1-800-832-8896**.

Possible Problems and Suggestions

Problem	Cause	Solution
Low yield	Incomplete Resuspension of Magnetic Particles	Thoroughly resuspend Mag-Bind® Particles CNR before use
	RNA Degraded during storage	Immediately process sample after collection or removal from storage
	SPR Wash Buffer not prepared correctly.	Prepare SPR Wash Buffer with the correct amount of ethanol.
	Inefficient cell lysis	Double the volume of Proteinase K added to the sample and extend incubation by 5 minutes.
	Cause	Solution
Problem with downstream applications	Insufficient RNA was used	• RNA in the sample already degraded, do not freeze and thaw the sample more than once or store at room temperature for too long • Quantify the purified DNA/RNA accurately and use sufficient DNA/RNA.
	Ethanol carry-over	Dry the Mag-Bind® Particles CNR completely before adding elution buffer.
Carryover of Magnetic Beads	Mag-Bind® Particles CNR would not fully magnetize on last step.	Place the eluted samples on a magnetic separation device for an additional 5 minutes or centrifuge at >4,000 x *g* for 5 minutes.

Ordering Information

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

Product	Part Number
E-Z 96® Magnetic Separation Device for 96-well microplates	MSD-01
500 µL Collection Plate, 5/25 preps	EZ9604-01/-02