omega BIO-TEK
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Product Manual

Mag-Bind® Viral DNA/RNA 96 Kit

M6246-01	1 x 96 preps
M6246-02	4 x 96 preps
M6246-03	12 x 96 preps

Manual Date: April 2024
Revision Number: v7.1

For Research Use Only

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Mag-Bind® Viral DNA/RNA 96 Kit

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Introduction

The Mag-Bind® Viral DNA/RNA Kit is designed for rapid and reliable isolation of viral DNA and RNA from cell-free samples such as serum, plasma, cell culture supernatant and other biological samples such as swabs, aspirates, etc. The Mag-Bind® paramagnetic bead technology is optimized for the recovery of low viral titer and provides high quality viral DNA or RNA suitable for direct use in most downstream applications such as RT-PCR, PCR and other enzymatic reactions. The extraction methodology is easily adaptable to various automated systems and can be scaled up or down depending on the amount of starting material used. The kit is not designed to separate cellular DNA from viral nucleic acids and cellular nucleic acid will be co-purified if present.

If using the Mag-Bind Viral DNA/RNA Kit for the first time, please read this booklet to become familiar with the procedure. The samples are first lysed under highly denaturing conditions, inactivating the RNases in TNA Lysis Buffer. Carrier RNA is added to the lysis buffer to enhance the binding of viral RNA to the magnetic beads, particularly for low viral titer samples. The lysate is mixed with Mag-Bind® Particles CNR along with isopropanol to bind viral nucleic acids to the magnetic beads. The viral nucleic acid-bound Mag-Bind® Particles are washed in two different wash buffers and eventually eluted in Nuclease-free Water.

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

New in this Edition:

April 2024:
- Addition of Warnings and Safety Information.

September 2020:
- A new protocol for NP Swabs, Aspirates and BAL samples has been added.

March 2020:
- Number of Carrier RNA vials has been adjusted.

October 2010:
- This manual has been edited for content and redesigned to enhance user readability.

Kit Contents

Product	M6246-01	M6246-02	M6246-03
Purifications	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	320 mL
VHB Buffer	22 mL	88 mL	242 mL
Carrier RNA	1 mg	4 mg	12 mg
Proteinase K Solution (40 mg/mL)	1.1 mL	4.4 mL	14 mL
SPR Wash Buffer	25 mL	100 mL	3 x 100 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 12 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. Store all other components at room temperature and away from bright light. During shipment or storage in cool ambient conditions, precipitates may form in some buffers. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Warnings

This kit is for research use only.

Please read all instructions carefully before using the kit.

Decontaminate and dispose of all potentially infectious materials in accordance with applicable local, state, and national regulations. Please refer to safety data sheets (SDSs) for information on disposal of different components included in this kit.

Safety Information

All chemicals and biological materials are potentially hazardous. Biological samples such as plasma, serum, tissues, body fluids, blood etc. are potentially infectious and must be treated as biohazardous materials. Conduct all work in properly equipped facilities following universal precautions and using appropriate personal safety equipment such as disposable gloves, lab coats, safety glasses etc. as required by policies and procedures outlined by your facility. Please refer to safety data sheets (SDSs) for information on safe handling, transport and disposal of different components included in this kit. SDSs are made available in PDF format on the product page at www.omegabiotek.com. Discard all waste in accordance with the local safety regulations.

Some of the buffers included in the product contain guanidine-based chaotropic agents, which can form highly reactive compounds when combined with bleach. DO NOT add bleach or acidic solutions to guanidine-containing waste. Please access the SDSs online for detailed information on the reagents.

Preparing Reagents

1. 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	308 mL

2. 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	400 mL
M6246-03	400 mL per bottle

3. 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.

Kit	Nuclease-free Water to be Added
M6246-01	1 mL
M6246-02	4 mL
M6246-03	12 mL

50 µL Sample Volume

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

Materials and Equipment to be Supplied by User:

- Vortexer
- Magnetic separation device for 96-well plate (Recommend Alpaqua, Cat# A000380)
- 96-well microplate capable of 500 µL
- 100% ethanol
- 100% isopropanol
- Optional: Sealing film

Before Starting:

- Prepare VHB Buffer, SPR Wash Buffer, and Carrier RNA according to Preparing Reagents section on Page 5.

1. Freshly prepare a TNA Lysis Buffer/Carrier RNA/100% isopropanol mastermix according to the table below:

Buffer	Volume
TNA Lysis Buffer	60 µL
Carrier RNA	2 µL
100% isopropanol	70 µL

2. Transfer 132 µL TNA Lysis Buffer/Carrier RNA/100% isopropanol mastermix to each well of a 96-well microplate (not provided).

3. Add 50 µL plasma or serum to each well. Vortex for 1 minute. If using frozen samples, thaw at room temperature and vortex or pipet up and down 20 times 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 5 µL Proteinase K Solution to each well. Vortex for 5 minutes.

5. 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.

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 5 for instructions.

9. Vortex for 1 minute.

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

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 5 for instructions.

14. Vortex 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 completely submerged in Nuclease-free Water.

21. Vortex 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 96-well microplate and seal with sealing film (not provided).

25. Store the DNA/RNA at -70°C.

200 µL Sample Volume

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

Materials and Equipment to be Supplied by User:

- Vortexer
- Magnetic separation device for 96-well plate (Recommend Alpaqua, Cat# A000380)
- 96-well deep-well plate capable of 2.0 mL
- 96-well microplate capable of 500 µL
- 100% ethanol
- 100% isopropanol
- Optional: Sealing film

Before Starting:

- Prepare VHB Buffer, SPR Wash Buffer, and Carrier RNA according to Preparing Reagents section on Page 5.

1. Freshly prepare a TNA Lysis Buffer/Carrier RNA/100% isopropanol mastermix according to the table below:

Buffer	Volume
TNA Lysis Buffer	240 µL
Carrier RNA	8 µL
100% isopropanol	280 µL

2. Transfer 528 µL lysis mastermix to each well of a 96-well deep-well plate (not provided).

3. Add 200 µL plasma or serum into each well. Vortex for 1 minute. If using frozen samples, thaw at room temperature and vortex or pipet up and down 20 times before proceeding to Step 5.

   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 10 µL Proteinase K Solution to each well. Vortex for 5 minutes.

5. 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.

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 5 for instructions.

9. Vortex 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 5 for instructions.

14. Vortex 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 plasticware and magnetic separation device used. The Mag-Bind® Particles CNR must be completely submerged in Nuclease-free Water.

21. Vortex 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 96-well microplate (not provided) and seal with sealing film (not provided).

25. Store the DNA/RNA at -70°C.

NP Swabs, Aspirates and BAL Samples

Protocol for extracting viral RNA from nasopharyngeal (NP) swabs, nasopharyngeal aspirates, and bronchoalveolar lavage (BAL) samples

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

Materials and Equipment to be Supplied by User:

- Centrifuge capable of at least 10,000*g*
- Oven or incubator capable of 65°C
- Vortexer
- Magnetic separation device for 96-well plate (Recommend Alpaqua, Cat# A000380)
- 96-well deep-well plate capable of 2.0 mL (Recommend VWR, Cat# 73520-476)
- 96-well microplate capable of 500 µL
- 100% ethanol
- 100% isopropanol
- 1x PBS
- Optional: Sealing film

Before Starting:

- Prepare VHB Buffer, SPR Wash Buffer, and Carrier RNA according to Preparing Reagents section on Page 5.
- Centrifuge capable of at least 10,000*g*, oven or incubator capable of 65°C and 1X PBS are for use with Nasopharyngeal swabs (dry) preparation.

1. Prepare the sample according to one of the methods listed below:

   A. Nasopharyngeal swabs (dry):
      - Add 300 µL 1x PBS (not provided) and 5 µL Proteinase K Solution to each swab.
      - Incubate at 56°C for 10-20 minutes with occasional mixing.
      - Centrifuge ≥10,000*g* for 30 seconds.

   OR

   B. Nasopharyngeal swabs, nasopharyngeal aspirates and bronchoalveolar lavage in Universal Transport Media (UTM) or Viral Transport Media (VTM):
      - Vortex the tubes containing the swab for 1 minute at maximum speed.

2. Transfer 200 µL of sample to a new 96-well deep-well plate (not provided).

3. Freshly prepare a TNA Lysis Buffer/Carrier RNA mastermix according to the table below:

Buffer	Volume
TNA Lysis Buffer	240 µL
Carrier RNA	1 µL

4. Transfer 241 µL lysis mastermix to each well of a 96-well deep-well plate.

5. Vortex for 1 minute. Let sit at room temperature for 5-10 minutes.

6. Prepare a 100% isopropanol/Mag-Bind® Particles CNR mastermix according to the table below:

Buffer	Volume
100% isopropanol	280 µL
Mag-Bind® Particles CNR	2 µL

7. Vortex for 10 minutes .

   Note: If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes.

8. 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.

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

10. Remove the plate from the magnetic separation device.

11. Add 350 µL VHB Buffer to each well.

    Note: VHB Buffer must be diluted with ethanol prior to use. Please see Page 5 for instructions.

12. Vortex for 1 minute.

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

13. 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.

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

15. Remove the plate from the magnetic separation device.

16. Add 350 µL SPR Wash Buffer to each well.

    Note: SPR Wash Buffer must be diluted with ethanol prior to use. Please see Page 5 for instructions.

17. Vortex for 1 minute.

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

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

20. Repeat Steps 15-19 for a second SPR Wash Buffer wash step.

Optional: Repeat Step 20 for a third SPR Wash Buffer step.

21. Leave the plate on the magnetic separation device for 5-10 minutes to completely air dry the Mag-Bind® Particles CNR. Remove any residual liquid with a pipettor.

22. Remove the plate from the magnetic separation device.

23. Add 50-100 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 completely submerged in Nuclease-free Water.

24. Vortex for 10 minutes.

    Note: If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes.

25. Place the plate on a 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.

26. Transfer the cleared supernatant containing purified DNA/RNA to a new 96-well microplate (not provided) and seal with sealing film (not provided).

27. Store the DNA/RNA 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 the magnetic particles 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 Solution 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.
	Insufficient washing	Increase washes to 3-5 minutes with constant vortexing and/or add 3rd wash with SPR Wash Buffer.
	Ethanol carryover	Dry the magnetic particles completely before adding elution buffer.
Carryover of Magnetic Particles	Magnetic particles not fully magnetized on last step.	Place the eluted samples on a magnetic separation device for an additional 5 minutes or centrifuge at >4,000*g* for 5 minutes.

For more purification solutions, visit **www.omegabiotek.com**

AVAILABLE FORMATS


Spin Columns

96-Well Silica Plates


Mag Beads

SAMPLE TYPES


Blood / Plasma

Plasmid


Cultured Cells


Plant & Soil


NGS Clean Up


Tissue


FFPE


Fecal Matter

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