omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

Mag-Bind® Viral RNA XPress Kit

M6219-384	4 x 96 preps
M6219-2304	24 x 96 preps

Manual Date: April 2024
Manual Revision: v1.2

For Research Use Only

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Introduction

Mag-Bind® Viral RNA Xpress Kit follows a magnetic bead-based approach for the rapid and reliable isolation of viral RNA from nasopharyngeal (NP) swab specimens that are dry or in viral transport media (VTM). The extraction methodology is easily adaptable to various automated systems and can also be scaled up or down depending on the amount of starting sample amount used. The kit utilizes the proven Mag-Bind® technology that enables the purification of high-quality nucleic acids that are free of proteins, nucleases, and other impurities. The purified nucleic acids are ready for direct use in downstream applications such as qPCR, RT-qPCR, and more.

If using the Mag-Bind® Viral RNA Xpress Kit for the first time, please read this manual to become familiar with the procedure. The samples are first lysed in TNA Lysis Buffer under highly denaturing conditions to inactivate the RNases and to preserve the integrity of viral RNA. Carrier RNA is added to the lysis buffer to enhance the binding of viral RNA to the magnetic beads and to maximize the recovery from low viral titer samples. The lysate is then mixed with Mag-Bind® Particles RQ along with isopropanol to bind viral nucleic acids to the magnetic beads. The viral nucleic acid-bound Mag-Bind® Particles RQ are washed twice in 80% ethanol and then eluted in Nuclease-free Water. Please note that the kit is not designed to separate cellular nucleic acids from viral nucleic acids, therefore cellular nucleic acids will be co-purified if present.

Note: This kit can also be used for viral DNA extraction. For protocols with other sample sources, please contact your Omega Bio-tek representative.

Important:

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

2. Kits include enough reagents for the specified number of preparations plus an additional 10% overage to ensure there is sufficient volume. Please be aware that the actual number of preparations may be lower due to pre-aliquoting of reagents, processing partial plates, and automation platform used etc. Additional reagents are available for purchase separately. Please visit the product page at www.omegabiotek.com or contact your Omega Bio-tek representative for more details and ordering information.

New in this Edition:

April 2024
- Addition of Warnings and Safety Information.

April 2022
- An important statement is included clarifying how the actual number of preparations is dependent on various factors and may be lower than the number of preparations specified with the kit.

Kit Contents

Product	M6219-384	M6219-2304
Purifications	4 x 96	24 x 96
TNA Lysis Buffer	110 mL	640 mL
RMP Buffer	100 mL	500 mL
Nuclease-free Water	60 mL	250 mL
Carrier RNA	1 mg	3 mg
Mag-Bind® Particles RQ	2.2 mL	13 mL
User Manual	✓	✓

Storage and Stability

All of the Mag-Bind® Viral RNA XPress 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. Carrier RNA should be stored at -10 to -30°C. 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 RMP Buffer with 100% isopropanol as follows and store at room temperature.

Kit	100% Isopropanol to be Added
M6219-384	100 mL
M6219-2304	500 mL

2. 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 at -20°C. Do no freeze-thaw the aliquots of Carrier RNA more than 3 times.

Kit	Nuclease-free Water to be Added
M6219-384	1 mL
M6219-2304	3 mL

Optional Protocol Modifications: Different Sample Types

The standard protocol can be modified for extraction with viscous saliva/sputum and BAL samples or stabilized saliva from collection devices. Refer to the sections below to determine which protocol to use for the different sample types.

For nasopharyngeal swabs (dry) or nasopharyngeal swabs, nasopharyngeal aspirates and bronchoalveolar lavage samples in Viral Transport Medium (VTM), refer to the protocol on Page 7.

1. Viscous saliva/sputum and BAL samples

   Note: The following protocol is based on CDC guidelines for treatment of viscous sputum specimens. Please visit *https://www.cdc.gov/coronavirus/2019-ncov/downloads/processing-sputum-specimens.pdf* for more information.

   a. Add 100 µL of freshly prepared DTT solution (500 mM) to 5 mL cold sterile 0.01 M PBS (pH 7.2) and vortex breifly.

      Note: DTT must be prepared fresh. Discard any unused DTT solution.

   b. Add an equal volume of diluted DTT/PBS solution and sputum specimen (e.g. 200 µL sputum + 200 µL DTT/PBS solution).
   c. Incubate at room temperature for up to 30 minutes with moderate shaking to liquify sample.
   d. Transfer 200 µL liquified sample to each well of a 96-well deep-well plate (not provided).
   e. Continue to Step 4 on Page 8 Mag-Bind® Viral RNA XPress Kit Protocol.

2. Stabilized saliva from collection devices

   a. Add 200 µL saliva from collection device to each well of a 96-well deep-well plate (not provided).
   b. Continue to Step 4 on Page 8 Mag-Bind® Viral RNA XPress Kit Protocol.

Mag-Bind® Viral RNA XPress Kit Protocol

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 mL (Recommend VWR, Cat# 73520-476)
- 96-well microplate capable of 500 µL (Recommend Omega Bio-tek, Cat# EZ9604-02)
- 80% ethanol
- 100% isopropanol
- 1X PBS
- Optional: Sealing film

Before Starting:

- Prepare RMP Buffer and Carrier RNA according to "Preparing Reagents" section on Page 5.
- Prepare 80% ethanol.
- Vortex Mag-Bind® Particles RQ to completely resuspend.

1. Select one of the following procotols for removing the viral particles depending on swab transport method.

   A. Universal Transport Media (UTM)/Viral Transport Media (VTM) Swabs: Vortex the swabs for 30 minutes.

   OR

   B. Dry Swabs: Submerge the swab in 1X PBS (not provided). Incubate at 56°C for 30 minutes with constant mixing. Centrifuge at 10,000*g* (or maxmimum speed) for 30 seconds.

2. Freshly prepare a mastermix of TNA Lysis Buffer and Carrier RNA according to the table below:

Buffer	Amount per Purification	Total Amount per 96-well Plate
TNA Lysis Buffer	240 µL	25.3 mL*
Carrier RNA	1 µL	105 µL*

* 10% excess volume has been calculated for a 96-well plate.

3. Transfer 200 µL UTM/VTM or PBS to each well of a 96-well deep-well plate (not provided).

4. Add 241 µL TNA Lysis Buffer/Carrier RNA mastermix to each sample. Vortex or pipet up and down 20 times.

5. Prepare a mastermix of 100% isopropanol and Mag-Bind® Particles RQ according to the table below:

Buffer	Amount per Purification	Total Amount per 96-well Plate
100% isopropanol	280 µL	30 mL*
Mag-Bind® Particles RQ	5 µL	530 µL*

*10% excess volume has been calculated for a 96-well plate.

6. Add 285 µL 100% isopropanol/Mag-Bind® Particles RQ mastermix. Pipet up and down 20 times.

   Note: Make sure Mag-Bind® Particles RQ are completely resuspended in mastermix before use.

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 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 from the magnetic separation device.

11. Add 350 µL RMP Buffer. Vortex for 5 minutes.

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

12. 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.

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

14. Add 350 µL 80% ethanol (not provided). Vortex for 5 minutes.

15. 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.

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

17. Repeat Steps 14-16 for a second 80% ethanol wash step.

18. Leave the plate on the magnetic separation device. Wait 1 minute. Remove residual liquid with a pipettor. Dry the Mag-Bind® Particles RQ for an additional 5-10 minutes.

19. Remove the plate from the magnetic separation device.

20. Add 50-100 µL Nuclease-free Water.

21. Vortex for 10 minutes.

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

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

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

24. Store RNA at -80°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**.

Problem	Cause	Solution
Low yield	Incomplete resuspension of magnetic particles	Resuspend the magnetic particles by vortexing before use.
	RNA degraded during storage	Immediately process sample after collection or removal from storage.
	Loss of magnetic particles during operation	Increase the particle collection/ magnetization time.
Problem	Cause	Solution
Problem with downstream applications	Ethanol carryover	Dry the magnetic particles completely before adding Nuclease-free Water.
	Insufficient RNA was used	RNA in the sample already degraded: do not freeze-thaw the sample more than once or store at room temperature for too long.
Carryover of magnetic particles	Carryover of magnetic particles in the eluted RNA will not effect downstream applications	To remove the carryover magnetic particles from the eluted RNA, simply magnetize the magnetic particles and carefully transfer the RNA eluate to a new tube or plate.

Notes:

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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