omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

Mag-Bind® PX Blood RNA 96 Kit

M7763-00	1 x 96 preps
M7763-01	4 x 96 preps

Manual Date: November 2018
Revision Number: v6.0

For Research Use Only

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Introduction

This Omega Bio-tek Mag-Bind® PX Blood RNA 96 Kit is designed to isolate total RNA including microRNA from up to 2.5 mL whole blood stabilized in PreAnalytiX® PAXgene® Blood RNA Tubes. Fresh, refrigerated, and frozen samples can be processed using the Mag-Bind® PX Blood RNA 96 Kit.

Overview

If using the Mag-Bind® PX Blood RNA 96 Kit for the first time, please read this booklet to become familiar with the procedure and its various modifications. The Mag-Bind® PX Blood RNA procedure begins with blood that is collected and mixed in PAXgene® Blood RNA Tubes. During sample collection blood cells are lysed and preserved for later RNA purifications. Blood is spun down and the crude RNA/DNA pellet is collected and washed. The pellet is resuspended and digested with proteinase K. Samples are transferred into a E-Z 96 PX Filter Plate and centrifuged briefly to obtain clear lysate. The Mag-Bind® Particles RQ are dispersed into the sample to bind RNA. After few wash steps, purified RNA is eluted with Nuclease-free Water. Purified RNA can be directly used in downstream applications without the need for further purification.

New in this Edition:

November 2018:
- DEPC Water has been replaced with Nuclease-free Water. DEPC Water is no longer provided in this kit.
- PR032 (DEPC Water, 100 mL) has been discontinued and is no longer available to purchase.

June 2018:
- DNase Digestion Buffer storage temperature changed from room temperature to -20°C.

November 2016:
- NTL Lysis Buffer has replaced PXR Buffer.
- NTL Lysis Buffer has replaced PXL Buffer and Mag-Bind® Particles RQ has replaced Mag-Bind® Particles CNR to increase yield and RIN value.
- Mag-Bind® DNase I and DNase Digestion Buffer are now included with kit and the standard protocol has been modified to include DNase digestion.

Kit Contents

Product	M7763-00	M7763-01
Purifications	1 x 96 preps	4 x 96 preps
E-Z 96 PX Filter Plate	1	4
Mag-Bind® Particles RQ	2.2 mL	8.8 mL
NTL Lysis Buffer	40 mL	150 mL
VHB Buffer	22 mL	88 mL
RNA Wash Buffer II	25 mL	100 mL
Nuclease-free Water	2 x 500 mL	3 x 1000 mL
Proteinase K Solution	4.4 mL	18 mL
Mag-Bind® DNase I	220 µL	4 x 220 µL
DNase Digestion Buffer	25 mL	2 x 25 mL
User Manual	✓	✓

Storage and Stability

All of the Mag-Bind® PX Blood RNA 96 Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® Particles RQ must be stored at 2-8°C. Mag-Bind® DNase I and DNase Digestion Buffer should be stored at -20°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. All remaining components should be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in NTL Lysis Buffer. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Preparing Reagents

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

Kit	100% Ethanol to be Added
M7763-00	28 mL
M7763-01	112 mL

2. Dilute RNA Wash Buffer II with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M7763-00	100 mL
M7763-01	400 mL

Important Notes

Please take a few minutes to read this booklet in its entirety to become familiar with the procedures.

- Whenever working with RNA, always wear gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents.

- Equilibrate samples and reagents to room temperature before beginning this protocol. All steps should be carried out at room temperature unless otherwise noted. Work quickly, but carefully.

- Prepare all materials required before starting the procedure to minimize RNA degradation.

Quantification and Storage of RNA

To determine the concentration and purity of RNA, measure absorbance at 260 nm and 280 nm with a spectrophotometer. One OD unit measured at 260 nm corresponds to 40 µg/mL RNA. Nuclease-free water is slightly acidic and can lower A_{260}/A_{280} ratios. Use TE buffer to dilute RNA prior to spectrophotometric analysis. The A_{260}/A_{280} ratio of pure nucleic acids is 2.0, while an A_{260}/A_{280} ratio of 0.6 denotes pure protein. A ratio of 1.8-2.0 corresponds to 90%-100% pure nucleic acid. Phenol has a maximum absorbance at 270 nm and can interfere with spectrophotometric analysis of DNA or RNA. Store RNA samples at -70°C in water. Under these conditions, RNA is stable for more than a year.

Integrity of RNA

It is highly recommended that RNA quality be determined prior to beginning all downstream applications. The quality of RNA can be best assessed by denaturing agarose gel electrophoresis with ethidium bromide staining. The ribosomal RNA bands should appear as sharp, clear bands on the gel. The 28S band should appear to be double that of the 18S RNA band (23S and 16S if using bacteria). If the ribosomal RNA bands in any given lane are not sharp and appear to be smeared towards the smaller sized RNA, it is very likely that the RNA undergone degradation during the isolation, handling, or storage procedure. Although RNA molecules less than 200 bases in length do not efficiently bind to the HiBind® matrix, a third RNA band, the tRNA band, may be visible when a large number of cells are used.

Mag-Bind® PX Blood RNA Kit Protocol - Total RNA

The following protocol is designed for isolating total RNA include microRNA from 2.5 mL whole blood preserved in PreAnalytiX® PAXgene® Blood RNA Tubes.

Materials and Equipment to be Supplied by User:

- Centrifuge capable of 3,000 x *g* with an adaptor for 96-well plates
- Centrifuge capable of 3,000 x *g* with proper adaptor for PAXgene® blood tubes
- Shaking incubator capable of 1,000 RPM and 55°C
- Magnetic Separation Device (MSD-02 for 1.5 mL tubes; MSD-01 for 96-well plates
- 2 mL deep-well plates and 1.5 mL centrifuge tubes
- 96-well microplate
- Sealing film
- Multichannel pipette
- Nuclease-free pipette tips
- 100% ethanol
- 100% isopropanol

Before Starting:

- Prepare Reagents according to the instructions on Page 4
- Heat incubator to 55°C

1. Collect blood directly into each PAXgene® Blood RNA Tube according to your laboratory's standard procedures. Immediately invert 15-20 times to ensure the sample and preservative are mixed and uniform.

2. Let sit at room temperature for a minimum of 2 hours. PAXgene® tubes are stable for up to 72 hours at room temperature.

3. Centrifuge at 3,000 x *g* for 10 minutes.

4. Aspirate and discard the supernatant.

5. Add 5 mL Nuclease-free Water. Vortex at maximum speed to completely resuspend the pellet.

6. Centrifuge at 3,000 x *g* for 10 minutes.

7. Aspirate and discard the supernatant. Invert the tube on paper towels for 3 minutes to remove residual liquid in the tube.

8. Add 265 µL Nuclease-free Water and 40 µL Proteinase K. Immediately vortex at maximum speed completely resuspend the pellet.

9. Add 225 µL NTL Lysis Buffer. Vortex at maximum speed to mix thoroughly.

10. Transfer the lysate to a new 1.5 mL microcentrifuge tube or 96-well deep-well plate.

11. Incubate at 55°C for 10 minutes with shaking at 1,000-1,400 RPM.

12. Place the E-Z 96® PX Filter Plate on top of a new 96-well deep-well plate.

13. Transfer the entire sample from Step 11 to the E-Z 96® PX Filter Plate.

14. Centrifuge at 3,000 x *g* for 3 minutes. Discard the E-Z 96® PX Filter Plate.

15. Add 80 µL NTL Lysis Buffer, 660 µL isopropanol, and 20 µL Mag-Bind® Particles RQ to each sample. Vortex at maximum speed for 60 seconds.

    Note: The Mag-Bind® Particles RQ will settle and clump together at the bottom of the bottle during storage. Vortex the Mag-Bind® Particles RQ thoroughly before use.

16. 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.

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

18. Remove the plate from the magnetic separation device.

19. Add 450 µL VHB Buffer to each sample.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

20. Resuspend the Mag-Bind® Particles RQ by vortexing or pipetting up and down 20 times.

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

21. 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.

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

23. Remove the plate from the magnetic separation device.

24. Add 500 µL RNA Wash Buffer II to each sample.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

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

26. 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.

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

28. For each sample, prepare the Mag-Bind® DNase I stock solution as follows:

Buffer	Volume per Prep
DNase I Digestion Buffer	98 µL
Mag-Bind® DNase I	2 µL
Total Volume	100 µL

Important Notes:

- Mag-Bind® DNase I is very sensitive and prone to physical denaturing. **Do not vortex the Mag-Bind® DNase I mixture.** Mix gently by inverting the tube.
- Freshly prepare Mag-Bind® DNase I stock solution right before RNA isolation.
- Standard DNase buffers are not compatible with Mag-Bind® DNase I digestion. The use of other buffers may affect the binding of RNA to the Mag-Bind® Particles RQ and may reduce RNA yields and purity.
- All steps must be carried out at room temperature. Work quickly, but carefully.

29. Remove the plate from the magnetic separation device.

    Note: It is very important to remove any residual liquid from the wells of the plate before adding the Mag-Bind® DNase I stock solution.

30. Add 100 µL Mag-Bind® DNase I stock solution to each sample.

31. Resuspend the Mag-Bind® Particles RQ by vortexing or pipetting up and down 20 times.

32. Let sit at room temperature for 15 minutes.

33. Add 75 µL RNA Wash Buffer II.

34. Resuspend the Mag-Bind® Particles RQ by vortexing or pipetting up and down 20 times.

35. Let sit at room temperature for 5 minutes.

36. 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.

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

38. Remove the plate from the magnetic separation device.

39. Add 500 µL RNA Wash Buffer II.

40. Resuspend the Mag-Bind® Particles RQ by vortexing or pipetting up and down 20 times.

41. Let sit at room temperature for 5 minutes.

42. 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.

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

44. Remove the plate from the magnetic separation device.

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

46. Add 50-100 µL Nuclease-free Water to each sample.

47. Resuspend the Mag-Bind® Particles RQ by vortexing or pipetting up and down 20 times.

    Note: Complete resuspension is required for efficient elution.

48. Let sit for 3 minutes at room temperature.

49. 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.

50. Transfer the cleared supernatant containing purified RNA to a clean 96-well microplate (not provided).

51. 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**.

Possible Problems and Suggestions

Problem	Cause	Solution
Low RNA Yield	Incomplete resuspension of Mag-Bind® Particles RQ	Resuspend the Mag-Bind® Particles RQ by vortexing before use.
	RNA degraded during sample storage	Make sure the sample is properly stored and make sure the samples are processed immediately after removal from storage.
	RNA Wash Buffer II was not prepared correctly	Prepare RNA Wash Buffer II by adding ethanol according to the instructions on Page 4.
	Loss of Mag-Bind® Particles RQ during operation	Increase the collection time.
	Undissolved particles in the cell lysate cause congregation of the Mag-Bind® Particles RQ	Make sure the lysate is clear of particles before adding the Mag-Bind® Particles RQ.
No RNA eluted	RNA Wash Buffer II was not prepared correctly	Prepare RNA Wash Buffer II by adding ethanol according to the instructions on Page 4.
Problems with downstream applications	Insufficient RNA was used	RNA in the blood sample is already degraded. Collect blood according to the PAXgene® protocol.
		Quantify the purified RNA accurately and use sufficient RNA.
Carryover of the Mag-Bind® Particles RQ during elution	Carryover of the Mag-Bind® Particles RQ in the eluted RNA will not effect downstream applications	To remove the carryover Mag-Bind® Particles RQ from eluted RNA, simply place the plate on the magnetic separation device and carefully transfer the samples to a new plate.
DNA contamination	Inefficient DNA removal	Make sure to use the proper starting material.

Ordering Information

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

Product	Part Number
Magnetic Separation Device for 1.5 mL Tubes	MSD-02
96-well Round-well Plate (1.2 mL), 10/pk	SSI-1780-00
96-well Round-well Plate (1.2 mL), 100/cs	SSI-1780-01
Multi-Channel Disposable Reservoirs, 100/pk	AC1331-01
SealPlate Film, 100/box	AC1200-01
Nuclease-free Water, 1000 mL	PD092
RNA Wash Buffer II, 20 mL	PDR046

Notes:

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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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