omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

Mag-Bind® Blood RNA 96 Kit

M2839-00	1 x 96 preps
M2839-01	4 x 96 preps

Manual Date: July 2019
Revision Number: v6.0

For Research Use Only

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Introduction and Overview

The Mag-Bind® Blood RNA 96 Kit is designed for rapid and reliable isolation of total and viral RNA from mammalian whole blood. The Mag-Bind® Particles technology provides high-quality RNA, which is suitable for direct use in most downstream applications, such as amplifications and enzymatic reactions. These protocols can be easily adapted to an automated system and the procedure can be scaled up or down.

If using the Mag-Bind® Blood RNA 96 Kit for the first time, please read this booklet to become familiar with the procedure and its various modifications. This kit is designed to isolate high quality RNA from fresh blood. Use of frozen blood is not recommended and can cause significant decline in RNA quality. This kit is not appropriate for whole blood preserved in PreAnalytiX® PAXgene® Blood RNA tubes, Tempus Blood RNA tubes or other stabilization media. Please contact the technical support staff at Omega Bio-tek for kit recommendations with blood preserved in stabilization media.

Samples are rapidly lysed in a specially formulated buffer containing detergent and chaotropic salt. The binding conditions are adjusted to capture the released nucleic acids onto the silica surface of the magnetic particles. The particles/nucleic acids complex is then magnetically separated to wash away the cellular debris and other contaminants. DNA contamination is eliminated with a Mag-Bind® DNase I treatment. RNA is purified from the Mag-Bind® DNase I reaction mixture using a second magnetic particle binding and washing procedure. Pure RNA is eluted in nuclease-free water and ready for use in various downstream applications without the need for further purification.

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:

July 2019:
- RBL Buffer has been replaced with TNA Lysis Buffer. RBL Buffer is no longer provided in this kit.
- RXT Wash Buffer has been replaced with VHB Buffer. RXT Wash Buffer is no longer provided in this kit.
- RNA Wash Buffer II has been replaced with user supplied 80% ethanol. RNA Wash Buffer II is no longer provided in this kit.

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.

Important Notes

Please take a few minutes to read this booklet in its entirety to become familiar with the procedures. Prepare all materials required before starting to minimize RNA degradation.

- Whenever working with RNA, always wear gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips and plastic ware for 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 slightly alkaline buffered solutions such as TE buffer to dilute RNA and as a blank prior to spectrophotometric analysis for more accurate measurements. 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 or by automated electrophoresis systems such as Agilent's Bioanalyzer and TapeStation instruments.

On an agarose gel, the ribosomal RNA bands should appear as sharp, clear bands. 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 underwent degradation during the isolation, handling, or storage procedure. Although RNA molecules less than 200 bases in length do not efficiently bind to the magnetic beads, a third RNA band, the tRNA band, may be visible when a large number of cells are used.

TapeStation and Bioanalyzer Systems provide objective measurement of RNA quality and assess the RNA intact-ness by assigning an RNA Integrity Number (RINe). RINe is presented as a value between 1 and 10, where 10 represents RNA sample of highest quality. Typically, RINe value greater than 7 is considered suitable for most downstream applications.

Kit Contents

Product	M2839-00	M2839-01
Purifications (200 µL blood)	1 x 96	4 x 96
Mag-Bind® Particles CNR	2.2 mL	8.8 mL
TNA Lysis Buffer	28 mL	120 mL
Proteinase K Solution	2.4 mL	9 mL
VHB Buffer	2 x 44 mL	3 x 88 mL
Nuclease-free Water	30 mL	60 mL
Mag-Bind® DNase I	225 µL	900 µL
DNase I Digestion Buffer	25 mL	2 x 25 mL
User Manual	✓	✓

Storage and Stability

All Mag-Bind® Blood RNA 96 Kit components are guaranteed for at least 12 months from the date of purchase when stored as recommended. Proteinase K Solution can be stored at room temperature for 12 months. For long-term storage, store Proteinase K Solution at 2-8°C. Mag-Bind® Particle CNR must be stored at 2-8°C. Mag-Bind® DNase I and DNase I Digestion Buffer must be stored at -20°C. Store all other components at room temperature (15-25°C). Check buffers for precipitates before use. Redissolve any precipitates by warming to 37˚C.

Preparing Reagents

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

Kit	100% Ethanol to be Added
M2839-00	56 mL per bottle
M2839-01	112 mL per bottle

Mag-Bind® Blood RNA 96 Kit Protocol - 200 µL blood

The following protocol is designed for isolating total RNA from 200 µL fresh whole blood. For best RNA quality, always use blood that has not been frozen. Frozen blood can be used with this protocol, however, RNA quality could be compromised as the result of the freeze-thaw process.

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:

- Magnetic separation device for 96-well microplate
- Nuclease-free 1 mL or 2 mL 96-well deep-well plate (Recommend Cat# EZ9602-01)
- Nuclease-free 96-well microplate (Recommend Cat# EZ9604-01)
- Multichannel pipette
- Nuclease-free pipette tips
- 100% ethanol
- 80% ethanol
- Sealing film

Before Starting:

- Prepare VHB Buffer according to the "Preparing Reagents" section on Page 6.

1. Add 260 µL TNA Lysis Buffer and 260 µL 100% isopropanol to each well of a 96-well deep-well plate.

2. Add 200 µL blood sample to each well and shake for 1 minute.

3. Add 20 µL Proteinase K Solution and 20 µL Mag-Bind® Particles CNR. Pipet up and down 10 times or shake for 5 minutes to mix thoroughly.

   Note: Proteinase K Solution must be added after the blood sample has been added to TNA Lysis Buffer. Mag-Bind® Particles CNR and Proteinase K Solution can be made as a mastermix.

4. Let sit at room temperature for 10 minutes.

5. 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.

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

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

8. Add 600 µL VHB Buffer. Pipet up and down 20 times or shake for 2 minutes to mix thoroughly.

   Note: VHB Buffer must be diluted with 100% ethanol before use. Please see Page 6 for instructions.

9. 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.

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

11. Repeat Steps 7-10 for a second VHB Buffer wash step.

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

13. Add 600 µL 80% ethanol (not provided). Pipet up and down 10 times or shake for 2 minutes to mix thoroughly.

14. 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.

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

16. Leave the plate on the magnetic separation device for 3 minutes to air dry the Mag-Bind® Particles CNR. Remove any residual liquid with a pipettor.

17. Prepare the Mag-Bind® DNase I digestion mix as detailed in the table below:

    Note: If total nucleic acid (DNA and RNA) is desired, skip Mag-Bind® DNase I digestion steps (Steps 18-24) and proceed to Step 25 for isolating both DNA and RNA.

Number of Samples	Mag-Bind® DNase I Digestion Buffer	Mag-Bind® DNase I	Total Volume
1	98 µL	2 µL	100 µL
4	431 µL*	8.8 µL*	439.8 µL
10	1078 µL*	22 µL*	1100 µL
96	10.35 mL*	211 µL*	10.56 mL

** Volumes are calculated 10% extra to offset pipetting error.*

Important Notes:

- Mag-Bind® DNase I is very sensitive and prone to physical denaturation. **Do not vortex the Mag-Bind® DNase I mixture.** Mix gently by shaking the plate.
- Freshly prepare Mag-Bind® DNase I digestion mix right before RNA isolation.
- All steps must be carried out at room temperature. Work quickly, but carefully.

18. Add 100 µL Mag-Bind® DNase I digestion mix. Pipet up and down 20 times or shake gently for 2 minutes to mix.

    Note: It is very important to remove any liquid drop from the wells before adding the Mag-Bind® DNase I digestion mix. Mag-Bind® DNase I digestion mix must be used immediately once it is prepared.

19. Let sit at room temperature for 10-15 minutes.

20. Add 400 µL 80% ethanol (not provided). Pipet up and down 20 times or shake for 5 minutes to mix.

21. Let sit at room temperature for 5 minutes.

22. 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.

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

24. Remove the plate containing the Mag-Bind® Particles CNR from the magnetic separation device.

25. Add 450 µL 80% ethanol (not provided). Pipet up and down 20 times or shake for 2 minutes to mix thoroughly.

26. 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.

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

28. 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.

29. Add 100 µL Nuclease-free Water. Pipet up and down 20 times or shake for 1 minute to mix thoroughly.

30. Let sit at room temperature for 3 minutes.

31. 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.

32. Transfer the cleared supernatant containing purified RNA into a new RNase-free microplate.

33. Store eluted RNA at -70°C.

Note: Any combination of the following steps can be used to help increase RNA yield.

- Heat the Nuclease-free Water to 70°C before adding to the beads.
- Increase the incubation time to 5 minutes.
- Increase the elution volume.
- Repeat the elution step with fresh Nuclease-free Water (this may increase the yield, but decrease the concentration).

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 RNA Yield	Incomplete resuspension of magnetic particles	Resuspend the magnetic particles by vortexing before use
	RNA degraded during sample storage	Make sure samples are stored properly and that the samples are processed immediately after collection or removal from storage
	Loss of magnetic particles during procedure	Increase the magnetic particle collection time
	Blood clots cause congregation of magnetic particles	Make sure the sample is clear of blood clots before adding magnetic particles.
Problem	Cause	Solution
No RNA eluted	VHB Buffer was not diluted with 100% ethanol	Prepare VHB Buffer by adding 100% ethanol according to the instructions
Problem	Cause	Solution
Problem with downstream applications	Insufficient RNA was used	RNA is already degraded; always use fresh blood for RNA isolation
		Quantify the purified RNA accurately and use sufficient RNA
Problem	Cause	Solution
Carryover of magnetic beads during elution	Carryover of magnetic particles in the eluted RNA will not effect downstream applications	To remove the carryover magnetic particles from eluted RNA, simply magnetize the magnetic particles and carefully transfer to a new plate
Problem	Cause	Solution
DNA contamination	Inefficient DNase I digestion	Make sure to use proper starting material
		Ensure that the DNase I digestion is carried out at room temperature

Ordering Information

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

Product	Part Number
96-well Square-well Plate (2.2 mL), 5/pk	EZ9602-01
96-well Round-well Plate (1.2 mL), 10/pk	7631-00
96-well Microplate (500 µL), 25/pk	EZ9604-02
Nuclease-free Water (1000 mL)	PD092

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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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