omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

Mag-Bind® FFPE RNA 96 Kit

M2551-00	1 x 96 preps
M2551-01	4 x 96 preps

Manual Date: March 2020
Revision Number: v6.1

For Research Use Only

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Mag-Bind® FFPE RNA 96

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Introduction

The Mag-Bind® FFPE RNA 96 Kit provides a rapid and reliable method for the isolation of total RNA from formalin-fixed, paraffin-embedded (FFPE) tissue sections. Nucleic acids in FFPE samples are heavily fragmented and often modified by formaldehyde due to fixation and embedding procedures. The specially formulated buffers in Mag-Bind® FFPE RNA 96 Kit are designed to minimize the effects of the formaldehyde modification and partially reverse cross-linking without the need for overnight digestion resulting in high-yielding, high-quality nucleic acids. Purified RNA is suitable for a variety of downstream applications including qRT-PCR, reverse transcription PCR, primer extension, expression array assays, microarray analyses, and next generation sequencing.

The Mag-Bind® FFPE RNA 96 Kit combines highly efficient binding properties of Mag-Bind® technology with a specially designed buffer system to isolate total RNA from FFPE samples. There are two protocols included in this manual — one that uses heat for paraffin removal and one that uses traditional xylene to remove paraffin from the sample. Samples are first lysed in RML Buffer aided by the presence of Proteinase K enzyme. The lysate is heated to denature the proteinases and reverse the chemical crosslinking of the nucleic acids. The lysate is then mixed with MFB Buffer and Mag-Bind® Particles SC to bind RNA to the magnetic particles. Post-binding genomic DNA is removed by DNase I digestion. After two rapid wash steps, purified RNA is eluted with Nuclease-free Water.

Starting Materials

Since standard formalin fixation and paraffin embedding procedures cause significant fragmentation of nucleic acids, we recommend following these guidelines to limit the extent of DNA/RNA fragmentation: 1) Use 4-10% formalin to fix tissue samples; 2) Limit the fixation time to 14-24 hours; 3) Completely dehydrate samples before embedding. Always use freshly cut sections of FFPE tissue for RNA isolation. For the first time user, we recommend using less than 3-5 sections of 10 µm thickness each. Depending on the yield and purity obtained, it may be possible to increase the starting material.

New in this Edition:

March 2020
- Storage and Stability section has been updated with change in GFC Buffer storage conditions.

July 2019
- DNase I Digestion Buffer has been renamed DNase Digestion Buffer. This is a name change only. The formulation has not changed.

February 2019
- DEPC Water has been replaced with Nuclease-free Water. DEPC Water is no longer provided in this kit.
- PR032 (DEPC Water, 100 mL) and 96-well Magnetic Separation Devices (MSD-01, MSD-01B) have been discontinued and are no longer available to purchase.

Kit Contents

Product Number	M2551-00	M2551-01
Preparations	1 x 96	4 x 96
Mag-Bind® Particles SC	2.2 mL	8.4 mL
RML Buffer	35 mL	140 mL
MFB Buffer	20 mL	80 mL
GFC Buffer	10 mL	40 mL
RNA Wash Buffer II	25 mL	2 x 50 mL
LPA Buffer	1.1 mL	4.4 mL
DNase Digestion Buffer	2 x 5 mL	2 x 25 mL
Mag-Bind® DNase I	150 µL	4 x 150 µL
Proteinase K Solution (20 mg/mL)	3 mL	12 mL
Nuclease-free Water	20 mL	40 mL
User Manual	✓	✓

Storage and Stability

All of the Mag-Bind® FFPE RNA 96 Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® DNase I and DNase Digestion Buffer should be stored at -20°C. Mag-Bind® Particles SC and LPA Buffer should be stored at 2-8°C for long-term use. 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. GFC Buffer is light sensitive, keep protected from light when not in use. All remaining components should be stored at room temperature. 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.

Preparing Reagents

Please take a few minutes to read this manual thoroughly to become familiar with the protocol before beginning the procedure. Prepare all materials required before starting to minimize RNA degradation. Wear gloves/protective goggles and take care when working with chemicals

1. Dilute RNA Wash Buffer II with 100% ethanol and store at room temperature.

Kit	100% Ethanol to be Added
M2551-00	100 mL
M2551-01	200 mL

2. Dilute GFC Buffer with 100% ethanol and store at room temperature.

Kit	100% Ethanol to be Added
M2551-00	20 mL
M2551-01	80 mL

Mag-Bind® FFPE RNA 96 Kit Protocol - Heat Method

Materials and Equipment to be Supplied by User:

- Nuclease-free 1.2 mL round-well plates
- Nuclease-free microplates
- Centrifuge with swing-bucket rotor capable of 4,000 x *g*
- RNase-free filter pipette tips
- Magnetic separation device for 96-well plates
- Water bath or heat block capable of 55°C
- Water bath or heat block capable of 80°C
- Water bath or heat block capable of 37°C
- 100% ethanol
- Sealing film

Before Starting:

- Prepare RNA Wash Buffer II and GFC Buffer according to the Preparing Reagents section on Page 4
- Set water bath or heat block to 55°C
- Set water bath or heat block to 80°C
- Set water bath or heat block to 37°C

1. Add 250 µL RML Buffer into each well of a 1.2 mL round-well plate.

2. Cut 2-5 paraffin sample sections between 5-10 µm to be placed in each well of the 96 plate. Note: Do not use the first 2-3 sections.

3. Immediately place 2-5 sections into each well of the round-well plate.

4. Centrifuge at 4,000 x *g* at room temperature for 2 minutes.

5. Incubate at 80°C for 15 minutes to melt the paraffin. Mix the sample a few times by gently shaking the tube. Make sure that the tissue sections stay submerged in the solution.

   Note: Seal the plate with sealing film to prevent evaporation during incubation.

6. Add 25 µL Proteinase K Solution (20 mg/mL). Incubate at 55°C for 15-30 minutes with occasional mixing. If necessary, extend the incubation to 1-3 hours or until the tissue is completely lysed.

7. Incubate at 80°C for 15 minutes.

8. Immediately centrifuge at 4,000 x *g* for 5 minutes. The paraffin will form a thin layer on top of the lysate solution.

9. Use a 1 mL pipette tip or large orifice tip to penetrate the paraffin layer, transfer 200 µL cleared lysate into a new round-well plate.

10. Add 200 µL MFB Buffer, 20 µL Mag-Bind® Particles SC, and 430 µL of 100% ethanol. Mix thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

    Note: If the RNA content from sample is expected low or miRNA is the target, then add 10 µL LPA Buffer.

11. Let sit at room temperature for 5-10 minutes.

12. Place the plate on a magnetic separation device for deep-well plates and wait 7-10 minutes or until the Mag-Bind® Particles SC are cleared from solution.

13. Aspirate and discard the cleared supernatant.

14. Remove the plate from the magnetic separation device.

15. Add 400 µL RNA Wash Buffer II. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

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

16. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

17. Aspirate and discard the cleared supernatant. Remove any liquid drops from each well.

18. Add 73.5 µL DNase Digestion Buffer and 1.5 µL RNase-free Mag-Bind® DNase I. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

19. Incubate at 37°C for 15 minutes.

20. Add 225 µL GFC Buffer. Mix thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

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

21. Let sit at room temperature for 3-5 minutes.

22. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

23. Aspirate and discard the cleared supernatant.

24. Remove the plate from the magnetic separation device.

25. Add 400 µL RNA Wash Buffer II. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

26. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

27. Aspirate and discard the cleared supernatant. Remove any liquid drops from tube.

28. Repeat Step 24-27 for a second RNA Wash Buffer II wash step.

29. Air dry the Mag-Bind® Particles SC by leaving the plate on the magnetic separation device for 10 minutes. Remove any residual liquid with a pipette.

30. Add 30-50 µL Nuclease-free Water. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 30 seconds or pipetting up and down 30 times.

31. Let sit at room temperature for 10 minutes.

32. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

33. Transfer the cleared supernatant containing purified RNA into a new nuclease-free 96-well microplate (not supplied) and seal with sealing film.

34. Store the purified RNA at -80°C.

Mag-Bind® FFPE RNA 96 Kit Protocol - Xylene Method

Note: The following protocol uses xylene to remove paraffin from the FFPE sample. Use fume hood and take proper protection during xylene extraction.

Materials and Equipment to be Supplied by User:

- 100% ethanol
- Xylene
- Nuclease-free 1.2 mL round-well plates
- Nuclease-free microplates
- Centrifuge with swing-bucket rotor capable of 4,000 x *g*
- RNase-free filter pipette tips
- Magnetic separation device for 96-well plates
- Water bath or heat block capable of 55°C
- Water bath or heat block capable of 80°C
- Water bath or heat block capable of 37°C
- Sealing film

Before Starting:

- Prepare RNA Wash Buffer II and GFC Buffer according to the Preparing Reagents section on Page 4
- Set water bath or heat block to 55°C
- Set water bath or heat block to 80°C
- Set water bath or heat block to 37°C

1. Add 1 mL xylene into each well of a 1.2 mL round-well plate.

2. Cut 2-5 paraffin sample sections between 5-10 µm.

   Note: Do not use the first 2-3 sections.

3. Immediately place 2-5 sections each well of the 1.2 mL round-well plate.

4. Let sit at room temperature for 2 minutes.

5. Mix thoroughly by vortexing for 20 seconds.

6. Centrifuge at 4,000 x *g* at room temperature for 5 minutes to pellet the tissue.

   Note: If the tissue does not form a tight pellet, centrifuge for an additional 3 minutes.

7. Carefully remove and discard the xylene without disturbing the pellet.

8. Add 1 mL 100% ethanol to each well. Mix thoroughly by vortexing for 20 seconds.

9. Centrifuge at 4,000 x *g* for 5 minutes to pellet the tissue sample. The pellet should appear opaque.

10. Carefully remove and discard the ethanol. Remove any liquid drops with a pipette.

11. Repeat Steps 8-10 for a second ethanol wash step.

12. Air dry the tissue pellet for 10-20 minutes.

    Note: It is critical to completely dry the sample before the Proteinase K digestion step. Ethanol residue will effect the efficiency of the Proteinase K digestion. If a vacuum oven is available, place the tube into the vacuum oven preset at 45°C for 10-30 minutes.

13. Add 250 µL RML Buffer and 25 µL Proteinase K Solution (20 mg/mL). Resuspend the pellet by vortexing or pipetting up and down 20 times.

14. Incubate at 55°C for 15 minutes.

15. Incubate at 80°C for 15 minutes.

16. Centrifuge at 4,000 x *g* at room temperature for 5 minutes.

17. Carefully transfer 200 µL cleared supernatant into a new round-well plate.

18. Add 200 µL MFB Buffer, 20 µL Mag-Bind® Particles SC, and 430 µL 100% ethanol. Mix thoroughly by vortexing or pipetting up and down 10-20 times.

    Note: If the RNA content from sample is expected low or miRNA is the target, add 10 µL LPA Buffer.

19. Let sit at room temperature for 5-10 minutes.

20. Place the plate on a magnetic separation device for deep-well plates and wait 7-10 minutes or until the Mag-Bind® Particles SC are cleared from solution.

21. Aspirate and discard the cleared supernatant.

22. Remove the plate from the magnetic separation device.

23. Add 400 µL RNA Wash Buffer II. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

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

24. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

25. Aspirate and discard the cleared supernatant. Remove any liquid drops from each well.

26. Add 73.5 µL DNase Digestion Buffer and 1.5 µL RNase-free Mag-Bind® DNase I. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

27. Incubate at 37°C for 15 minutes.

28. Add 225 µL GFC Buffer. Mix thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

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

29. Let sit at room temperature for 3-5 minutes.

30. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

31. Aspirate and discard the cleared supernatant.

32. Remove the plate from the magnetic separation device.

33. Add 400 µL RNA Wash Buffer II. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

34. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

35. Aspirate and discard the cleared supernatant. Remove any liquid drops from the wells.

36. Repeat Step 32-35 for a second RNA Wash Buffer II wash step.

37. Air dry the Mag-Bind® Particles SC by leaving the plate on the magnetic separation device for 10 minutes. Remove any residual liquid with a pipette.

38. Add 30-50 µL Nuclease-free Water. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 30 seconds or pipetting up and down 30 times.

39. Let sit at room temperature for 10 minutes.

40. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

41. Transfer the cleared supernatant containing purified RNA into a new nuclease-free 96-well microplate (not supplied) and seal with sealing film.

42. Store the purified RNA at -80°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact our technical support staff, toll free, at **1-800-832-8896.**

Possible Problems and Suggestions

Troubleshooting Guide		
Problem	**Likely Cause**	**Suggestions**
Low RNA yields	Incomplete resuspension of magnetic particles	Resuspend the magnetic particles 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 collection or 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 magnetic particles during operation	Increase the collection time for magnetic particles.
	GFC Buffer not diluted with ethanol	Prepare GFC Buffer by adding ethanol according to the instructions on Page 4.
Problem with downstream application	Degraded RNA	During incubation at 37°C, do not incubate sample over 15 minutes.
Carryover of the magnetic beads in the elution	Carryover of the 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 plate.

Notes:

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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