omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

Mag-Bind® FFPE DNA 96 Kit

M6958-00	1 x 96 preps
M6958-01	4 x 96 preps

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For Research Use Only

Omega Bio-tek, Inc.
400 Pinnacle Way, Suite 450
Norcross, GA 30071
www.omegabiotek.com

770-931-8400
770-931-0230
info@omegabiotek.com

omega-bio-tek
omegabiotek
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Introduction

The Mag-Bind® FFPE DNA 96 kits provide a rapid and easy method for the isolation of total DNA from formalin-fixed, paraffin-embedded (FFPE) tissue sections. Due to fixation and embedding procedures, nucleic acids in FFPE samples are heavily fragmented and modified by formaldehyde. While the Mag-Bind® FFPE DNA 96 kits are optimized to minimize the effect of the formaldehyde modification, it is not recommended to use the DNA purified with these kits for downstream applications that require full length DNA.

Overview

The Mag-Bind® FFPE DNA 96 kits combine the high efficiency binding properties of Mag-Bind® technology with a specially designed buffer system to isolate total DNA sample from FFPE samples. There are two protocols included in this manual. The standard protocol uses a heating step to remove paraffin from the sample. The alternative protocol uses the traditional xylene extraction to remove paraffin.

After the paraffin removal steps, samples are first lysed in FTL2 Buffer with digestion of Proteinase K. The lysate is then heated to denature the proteinase and mixed with MB3 Buffer and magnetic particles to bind the nucleic acid on the surface of the Mag-Bind® particles. After two wash steps, purified DNA is eluted with Elution Buffer or nuclease-free water.

New in this Edition:

January 2018

- RNase A is no longer supplied with this kit. An optional RNase A digestion step has been added.

March 2015

- MB3 Buffer has been replaced with MB4 Buffer
- Mag-Bind® Particles CNR have been replaced with Mag-Bind® Particles CH
- 10 minute drying step has been replaced with a water wash step.

Kit Contents

Mag-Bind® FFPE DNA 96 Kit	M6958-00	M6958-01
Preparations	1 x 96 preps	4 x 96 preps
Mag-Bind® Particles CH	3.3 mL	14 mL
FTL2 Buffer	30 mL	110 mL
MB4 Buffer	75 mL	250 mL
MPW Buffer	30 mL	125 mL
DNA Wash Buffer	40 mL	160 mL
LPA	1.1 mL	4.4 mL
Proteinase K Solution	2.2 mL	9 mL
Elution Buffer	11 mL	44 mL
User Manual	✓	✓

Preparing Reagents

Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M6958-00	160 mL
M6958-01	640 mL

Dilute MPW Buffer with 100% isopropanol as follows and store at room temperature.

Kit	Isopropanol to be Added
M6958-00	30 mL
M6958-01	125 mL

Mag-Bind® FFPE DNA Protocol - 96-well plate without xylene

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of ≥4,000 x *g*
- Magnetic separation device for 96-well plates
- Water bath or heat block capable of 70°C
- Water bath or heat block capable of 80°C
- Vortexer
- 100% isopropanol
- 100% ethanol

Before Starting:

- Prepare Buffers according to Preparing Reagents section on Page 3.
- Set water baths or heat blocks to , 80°C, and 70°C.
- Vortex the Mag-Bind® Particles CH thoroughly before use.

1. Add 250 µL FTL2 Buffer into a new 96-well plate with a capacity of atleast 800 µL.

2. Cut 3-8 paraffin sample sections between 5-10 µm.

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

3. Immediately add 2-5 sections to the FTL2 Buffer.

4. Centrifuge at maximum speed (≥4,000 x *g)* for 5 minutes.

5. Incubate at 80°C for 15 minutes. Mix the sample a few times by gently vortexing the plate for 15 seconds. Make sure that the tissue sections stay submerged in the solution.

6. Let sit at room temperature for 5 minutes.

7. Add 20 µL Proteinase K Solution.

8. Incubate at 70°C for 3-5 hours with occasional mixing. If necessary, extend the incubation to overnight or until the tissue is completely lysed.

Optional: If RNA-free genomic DNA is required, add 10 µL RNase A (20 mg/mL, not provided) and let sit for 5 minutes at room temperature.

9. Centrifuge at maximum speed (>4,000 x *g)* for 5 minutes. The paraffin will form a thin layer on top of the lysate solution.

10. Transfer 200 µL cleared lysate into a new 96-well plate with a volume of atleast 1.0 mL.

    Tip: Use a 1 mL pipette tip or large orifice tip to penetrate the paraffin layer.

11. Add 500 µL MB4 and 30 µL Mag-Bind® Particles CH. Mix thoroughly by vortexing or pipetting up and down 10-20 times.

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

13. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

15. Remove the plate containing the Mag-Bind® Particles CH from the magnetic separation device.

16. Add 400 µL MPW Wash Buffer. Resuspend the Mag-Bind® Particles CH by vortexing or pipetting up and down 20 times.

    Note: MPW Wash Buffer must be diluted with isopropanol prior to use. Please see Page 3 for instructions.

17. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

18. Aspirate and discard the cleared supernatant. Remove any liquid drops from each well. Do not disturb the Mag-Bind® Particles CH.

19. Remove the plate containing the Mag-Bind® Particles CH from the magnetic separation device.

20. Add 400 µL DNA Wash Buffer. Resuspend the Mag-Bind® Particles CH by vortexing or pipetting up and down 20 times.

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

21. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

22. Repeat Steps 19-21 for a second DNA Wash step.

23. Place the plate on a magnetic separation device. Add 400 µL Nuclease Free Water. Immediately aspirate and discard the Nuclease Free Water.

    Note: Do not let the Nuclease-free Water remain on the Mag-Bind Particles CH for more than 60 seconds.

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

25. Add 30-50 µL Elution Buffer. Resuspend the Mag-Bind® Particles CH by vortexing or pipetting up and down 30 times.

26. Incubate at room temperature for 5 minutes.

27. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

28. Transfer the cleared supernatant containing purified DNA to a new 96-well plate. Store the DNA at -20°C.

Mag-Bind® FFPE DNA 96 - 96-well plate with xylene

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:

- Centrifuge with swing-bucket rotor capable of 4,000 x *g*
- Rotor adaptor for 96-well deep-well plates
- Magnetic separation device for 96-well deep-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 90°C
- Vortexer
- 1.2 mL or 2.0 mL round-well plates
- Nuclease-free 96-well microplates
- 100% isopropanol
- 100% ethanol
- Xylene
- Sealing film

Before Starting:

- Prepare Buffers according to Preparing Reagents section on Page 3.
- Set water baths or heat blocks to 90°C, 80°C, and 55°C.
- Vortex the Mag-Bind® Particles CH thoroughly before use.

1. Add 1 mL xylene into each well of a 1.2 mL or 2.0 mL round-well plate.

2. Cut 3-8 paraffin sample sections between 5-10 µm.

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

3. Immediately add 2-5 sections to the xylene. Vortex for 20 seconds to mix thoroughly.

4. Centrifuge at 4,000 x *g* for 5-10 minutes to pellet the tissue.

   Note: If the tissue does not form a tight pellet, centrifuge for an additional 5 minutes.

5. Aspirate and discard the xylene. Do not disturb the tissue pellet.

6. Add 1 mL 100% ethanol. Vortex for 20 seconds to mix thoroughly.

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

8. Aspirate and discard the ethanol. Do not disturb the tissue pellet. Remove any liquid drops with a pipette.

9. Repeat Steps 6-8 for a second ethanol wash step.

10. Let sit at room temperature for 10-20 minutes.

    Note: It is critical to completely dry the sample before the next Proteinase K digestion step. Residual ethanol will affect the efficiency of the Proteinase K digestion. If a vacuum oven is available, place the plate in the vacuum oven preset at 45°C for 10-20 minutes.

11. Add 250 µL FTL2 Buffer and 20 µL Proteinase K Solution. Resuspend the pellet by vortexing or pipetting up and down 20 times.

12. Incubate at 55°C for 3-5 hours with occasional mixing. If necessary, extend the incubation to overnight or until the tissue is completely lysed.

13. Incubate at 90°C for 45-60 minutes.

Optional: If RNA-free genomic DNA is required, add 10 µL RNase A (20 mg/mL, not provided) and let sit for 5 minutes at room temperature.

14. Centrifuge at 4,000 x *g* for 5 minutes.

15. Transfer 200 µL cleared supernatant into a new 1.2 mL or 2.0 mL round-well plate.

16. Add 500 µL MB4 Buffer and 30 µL Mag-Bind® Particles CH. Mix thoroughly by vortexing or pipetting up and down 10-20 times.

    Note: If DNA content from sample is expected to be low, add 10 µL LPA.

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

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

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

20. Remove the plate from the magnetic separation device.

21. Add 400 µL MPW Wash Buffer. Resuspend the Mag-Bind® Particles CH by vortexing or pipetting up and down 20 times.

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

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

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

24. Remove the plate from the magnetic separation device.

25. Add 400 µL DNA Wash Buffer. Resuspend the Mag-Bind® Particles CH by vortexing or pipetting up and down 20 times.

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

26. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

27. Repeat Steps 24-26 for a second DNA Wash step.

28. Place the plate on a magnetic separation device. Add 400 µL Nuclease Free Water. Immediately aspirate and discard the Nuclease Free Water.

    Note: Do not let the Nuclease Free Water remain on the Mag-Bind Particles CH for more than 60 seconds.

29. Remove the plate containing the Mag-Bind® Particles CH from the magnetic separation device.

30. Add 30-50 µL Elution Buffer. Resuspend the Mag-Bind® Particles CH by vortexing or pipetting up and down 30 times.

31. Incubate at room temperature for 5 minutes.

32. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

33. Transfer the cleared supernatant containing purified DNA to a clean 96-well plate. Store the DNA at -20°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 DNA yields	Incomplete resuspension of magnetic particles	Resuspend the magnetic particles by vortexing before use.
	DNA 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.
	MPW Buffer and DNA Wash Buffer were not prepared correctly	Prepare MPW Buffer and DNA Wash Buffer according to the instructions on Page 5.
	Loss of magnetic beads during operation	Increase the bead collection time.
Problem	Cause	Solution
Problem with downstream application	DNA is over fixated during tissue formalin fixation	Extend incubation time at 90°C to 90 minutes.
Carryover of the magnetic beads in the elution	Carryover the magnetic beads in the eluted DNA will not effect downstream applications	To remove the carryover magnetic particles from the eluted DNA, simply magnetize the magnetic particles and carefully transfer the DNA eluate to a new plate.

Ordering Information

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

Product	Part Number
SealPlate Film, 100/box	AC1200-01
96-well Microplate (500 µL), 5/pk	EZ9604-01
96-well Microplate (500 µL), 25/pk	EZ9604-02
DNA Wash Buffer, 40 mL	PS001
DNA Wash Buffer, 100 mL	PDR044
Elution Buffer, 100 mL	PDR048

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

AVAILABLE FORMATS


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96-Well Silica Plates


Mag Beads

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Plasmid


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NGS Clean Up


Tissue


FFPE


Fecal Matter

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Omega Bio-tek, Inc.
400 Pinnacle Way, Suite 450
Norcross, GA 30071
www.omegabiotek.com

770-931-8400
770-931-0230
info@omegabiotek.com

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