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Revised October 2009

Introduction

Mag-Bind® FFPE DNA Kit provides 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 Kit is optimized to minimize the effect of the formaldehyde modification, it is not recommended to use the DNA purified with this kit for downstream applications that requires full length DNA.

Principle

The Mag-Bind FFPE DNA Kit combines high efficient binding properties of Mag-Bind technology with specialized buffer system and unique process to isolate total DNA from FFPE sample. There are two protocols included in this manual. The standard protocol uses a heating step instead of xylene to remove paraffin from FFPE sample. The alternative protocol uses traditional xylene extraction to remove paraffin. After the paraffin removal step, samples are first lysed in FTL Buffer with digestion of Proteinase K. The lysate is then heated to denature the proteinase and mixed with MSL Buffer and magnetic particles to bind the nucleic acid on the surface of Mag-Bind particles. After two quick wash steps, purified DNA is eluted with DNA Elution Buffer or nuclease-free water.

Storage and Stability

Most components in the Mag-Bind® FFPE DNA Kit should be stored at room temperature . Once dissolved, the Proteinase K solution should be stored at -20°C. The Mag-Bind particles should be stored at 4-8°C for long term storage. During shipping and storage, crystals may form in the FTL and MSL Buffer, simply warm to 37°C to dissolve. All kit components are guaranteed for at least 12 months from the date of purchase.

Kit Contents

Product Number	M6958-00	M6958-01	M6958-02
Purification	1 x 96	4 x 96	20 x 96
Mag-Bind Particles R	2.2 ml	8.4 ml	42 ml
FTL Buffer	30 ml	100 ml	500 ml
MSL Buffer	30 ml	100ml	500 ml
MPW Buffer	25 ml	100 ml	500 ml
DNA Wash Buffer	25 ml	100 ml	2 x 200 ml
LPA	1.1 mL	4.4 mL	22.0 mL
Proteinase K	60 mg	240 mg	5 x 240 mg
Proteinase Storage Buffer	3 ml	11 ml	55 ml
Elution Buffer	10 ml	40 ml	200 ml
Instruction Manual	1	1	1

Before Starting

Important	
	<p>1. DNA Wash Buffer must be diluted with absolute ethanol before use and then store at room temperature.</p> <p>M6958-00 Add 100 ml absolute ethanol</p> <p>M6958-01 Add 400 ml absolute ethanol</p> <p>M6958-02 Add 800 ml absolute ethanol</p>
	<p>2. MPW Buffer must be prepared by adding one vial of RNase A and isopropanol before use and then store at room temperature.</p> <p>M6958-00 Add 25 ml Isopropanol and 1 vial of RNase A</p> <p>M6958-01 Add 100 ml Isopropanol and 1 vial of RNase A</p> <p>M6958-02 Add 500 ml Isopropanol and 1 vial of RNase A</p>
	<p>3. Proteinase K should be dissolved using Proteinase Storage Buffer and store the dissolved Proteinase K at -20°C.</p> <p>M6958-00 Add 2.4 ml Proteinase Storage Buffer</p> <p>M6958-01 Add 9.6 ml Proteinase Storage Buffer</p> <p>M6958-02 Add 9.6 ml Proteinase Storage Buffer</p>

Important Notes

Please take a few minutes to read this booklet thoroughly and become familiar with the protocol.

- **Prepare MPW Wash Buffer/RNase A:** Transfer RNase solution from its container and mix into MPW Wash Buffer (diluted with isopropanol).
- Under cool ambient conditions, crystals may form in FTL Buffer and MSL Buffer. This is normal and the bottle should be warmed to 37 °C to redissolve the precipitation.
- All centrifugation steps must be carried out at 22°C-25°C.

Starting Materials

Since standard formalin fixation and paraffin-embedding procedures cause significant fragmentation of nucleic acids. We recommend following the guidelines to limit the extent of DNA/RNA fragmentation: 1). Use 4-10% formalin to fixate 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 to use less than 3-5 sections with thickness of 10µM. Depending on the yield and purity obtained, it may be possible to increase the starting material.

Mag-Bind® FFPE DNA Protocol

Materials and Equipments supplied by User

- 96-100% ethanol (Do not use denatured alcohol)
 - Nuclease-Free 96 round-well plate or 1.5 ml tube
 - Centrifuge with swing-bucket rotor capable of 4000 x g
 - Sealing film
 - RNase-free filter pipette tips
 - Magnetic Separation device for 96-well plate (MSD-01) or 1.5 ml tube (MSD-02)
 - 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
 - Isopropanol
1. **Cut 2-5 Paraffin sample sections 5-10 µM thick.** Note: Do not use first 2-3 sections.
 2. **Pipette 250 µl FTL Buffer** into each well of 1.2 ml round well plate or 1.5 ml tube to be used.
 3. **Immediately place the 2-5 sections** into the wells of round well plate or 1.5 ml tube containing 250µl of FTL Buffer.
 4. For 1.2 ml round-well plate, centrifuge at 4,000 x g at room temperature for 5 minutes. For 1.5 ml tube, centrifuge at maximum speed ($\geq 14,000$ x g) for 60 seconds.
 5. **Incubate at 80°C for 15 minutes to melt the paraffin.** Mix the sample a few times by gently shaking the plate 2-3 times. Make sure that the tissue sections stay submerged in the solution.
Note: Seal the plate with sealing film to prevent evaporation during the incubation.
 6. **Add 20 µl Proteinase K to each sample. Incubate 3-5 hours or at 55°C with occasional mixing.** If necessary, extend the incubation to overnight or until tissue is completely lysed.
 7. **Incubate at 90°C for 45-60 minutes.**
 8. **Then immediately centrifuge at 4,000 x g (for plate) or maximum speed**

($\geq 14,000$ 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 96 round well plate or 1.5 ml tube.
10. **Pipette 200 µl MSL Buffer, 20 µl Mag-Bind Particle R and 420 µl isopropanol to each sample.** Mix thoroughly by vortexing or pipetting up and down 10-20 times. Incubate at room temperature for 5-10 minutes.

Note: If the DNA content from sample is expected low, Add 10 µL of LPA into each sample.

11. **Place the plate or tube onto a magnet and wait 7-10 minutes to collect magnetic beads.**

Note: If using MSD-01 magnet stand, a 500 µl Process Plate (EZ960-01/02) should be required for the rest of the protocol. Since the total volume of the sample is around 850 µl, this magnet stand requires to transfer sample into a 500 µl Process Plate twice (425 µl each) to process whole sample.

12. **Aspirate and discard the cleared supernatant.**
13. **Remove the plate or tube from the magnetic separation device. Add 400 µl MPW Wash Buffer into each well.** Resuspend the Mag-Bind Particles by pipetting up and down 20 times.
14. **Place the plate or tube onto magnet separation device to magnetize the magnetic particles.** Wait 5-7 minutes or until all the magnetic particles are cleared from the solution.
15. **Aspirate and discard the cleared supernatant.** Remove any liquid drops.
16. **Place the plate or tube onto a magnet separation device to magnetize the magnetic particles.** Wait 5-7 minutes or until all the magnetic particles are cleared from the solution.
17. **Aspirate and discard the cleared supernatant.**
18. **Remove the plate from the magnetic separation device. Add 400 µl DNA Wash Buffer into each well.** Resuspend the Mag-Bind Particles by pipetting

up and down 20 times.

19. **Place the plate or tube onto magnet separation device to magnetize the magnetic particles.** Aspirate and discard the cleared supernatant.
20. **Wash the Mag-Bind Particles with another 400 µl DNA Wash Buffer by repeating step 18-19.**
21. Aspirate and discard all the liquid from each well or tube. **Air dry the Mag-Bind particles by leaving the plate on the magnetic separation device for 5-10 minutes.** Remove any liquid drops with pipettor.
22. **Add 30-50 µl Elution buffer (supplied, 10mM Tris-HCl, pH 8.5) or nuclease-free water (not supplied) into each well.** Resuspend the Mag-Bind particles by pipetting up and down 30 times. Incubate at room temperature for 10 minutes.
23. Place the plate onto magnet separation device to magnetize the magnetic particles.
24. **Transfer the cleared supernatant containing purified DNA into a new nuclease-free 96-well microplate or 1.5 ml tube.** Store the purified DNA at -20°C.

Mag-Bind® FFPE DNA Protocol Using Xylene

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

Materials and Equipments supplied by User

- 96-100% ethanol (Do not use denatured alcohol)
- Xylene
- Nuclease-Free 96 round-well plate or 1.5 ml tube
- Centrifuge with swing-bucket rotor capable of 4000 x g
- Sealing film
- RNase-free filter pipette tips
- Magnetic Separation device 96-well plate (MSD-01) or 1.5 ml tube (MSD-02)
- 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
- Isopropanol

1. **Cut 2-5 Paraffin sample sections 5-10 µM thick.** Note: Do not use first 2-3 sections.
2. **Pipette 1 ml of xylene into each well of 1.2 ml round well plate or 1.5 ml tube to be used.**
3. **Immediately place the 2-5 sections into the wells of round well plate containing 1 ml xylene.**
4. **Mix thoroughly by vortexing for 20 seconds.**
5. **Centrifuge at 4000 x g or maximum speed (for 1.5 ml tube) at room temperature for 5-10 minutes to pellet the tissue.**
Note: If the tissue does not form a tight pellet, centrifuge additional 3 minutes.
6. Carefully remove the xylene without disturbing the pellet. Discard the xylene.
7. **Add 1 ml absolute ethanol (100%) to each well.** Mix thoroughly by vortexing for 20 seconds.
8. Centrifuge at 4000 x g or maximum speed (for 1.5 ml tube) for 5 minutes to pellet the tissue sample. The pellet should appear opaque.

9. Carefully remove and discard the ethanol. **Add another 1 ml absolute ethanol** to each sample. Resuspend pellet by vortexing for 20 seconds.
10. Centrifuge at 4000 x g or maximum speed (for 1.5 ml tube) for 5 minutes at room temperature.
11. Carefully remove and discard the ethanol. Remove any liquid drops with pipettor
12. **Air dry the tissue pellet for 10-20 minutes.**

Note: It is critical to completely dry the sample before next Proteinase K digestion step. Ethanol residue will effect the efficiency of Proteinase K digestion. If vacuum oven is available, place the plate into the vacuum oven preset at 45°C for 10-30 minutes
13. Add 250 µl FTL Buffer followed by 20 µl proteinase K solution (20mg/ml). Completely resuspend the pellet by vortexing or pipetting up and down for 20 times.
14. **Incubate the plate at 55°C for 3 hours or overnight.**
15. **Incubate the sample at 90°C for 30-60 minutes.**
16. Centrifuge at 4,000 x g or maximum speed (for 1.5 ml tube) for 5 minutes at room temperature.
17. Carefully transfer 200 µl of cleared supernatant into a new 96 round well plate or 1.5 ml tube.
18. **Pipette 200 µl MSL Buffer, 20 µl Mag-Bind Particle R and 420 µl isopropanol to each sample.** Mix thoroughly by vortexing or pipetting up and down 10-20 times. Incubate at room temperature for 5-10 minutes.

Note: If the DNA content from sample is expected low, add 10 µL of LPA into each sample.
19. **Place the plate or tube onto a magnet wait 5-7 minutes to collect magnetic beads. Remove and discard the cleared supernatant.**

Note: If using MSD-01 magnet stand, a 500 µl Process Plate (EZ960-01/02) should be required for the rest of the protocol. Since the total volume of the sample is around 850 µl, this magnet stand requires to transfer sample into a 500 µl Process Plate twice (425 µl each) to process whole sample
20. **Remove the plate or tube from the magnetic separation device. Add 400 µl MPW Wash Buffer into each well.** Resuspend the Mag-Bind Particles by pipetting up and down 20 times.
21. **Place the plate or tube onto magnet separation device to magnetize the magnetic particles.** Wait 7-10 minutes or until all the magnetic particles are cleared from the solution.
22. **Aspirate and discard the cleared supernatant.** Remove any liquid drops.
23. **Add 400 µl DNA Wash Buffer.** Resuspend the Mag-Bind Particles by pipetting up and down 20 times.
24. **Place the plate or tube onto magnet separation device to magnetize the magnetic particles.** Aspirate and discard the cleared supernatant.
25. **Wash the Mag-Bind Particles with another 400 µl DNA Wash Buffer by repeating step 23-24.**
26. Aspirate and discard all the liquid. **Air dry the Mag-Bind particles by leaving the plate on the magnetic separation device for 10 minutes.** Remove any liquid drops.
27. **Add 30-50 µl Elution Buffer (supplied) or nuclease-free water (not supplied) into each well.** Resuspend the Mag-Bind particles by pipetting up and down 30 times. Incubate at room temperature for 10 minutes.
28. Place the plate or tube onto magnet separation device to magnetize the magnetic particles.
29. **Transfer the cleared supernatant containing purified DNA into a new nuclease-free 96-well microplate(not supplied).** Store the purified DNA at -20°C.

Trouble Shooting Guide

Low DNA Yield	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 RWB Buffer by adding isopropanol or ethanol according to instruction
	Loss of magnetic beads during operation	Increase the beads collection time
No DNA Eluted	MPW Wash Buffer Concentrate not diluted with Isopropanol	Prepare MPW Buffer as instructed on the label.
	DNA Wash Buffer were not prepared correctly	Prepare DNA Wash Buffer by adding ethanol according to instruction
Problem with downstream application	DNA is over fixate during Tissue formalin fixate	Extend incubation time at 90°C to 90 minutes.
Carryover of the magnetic beads in the elution	Carryover from the magnetic beads in the eluted DNA will not effect downstream applications	To remove the carryover magnetic beads from eluted DNA, simply magnetize the magnetic beads and carefully transfer to a new tube or plate.

Note