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Introduction

Mag-Bind RNA Kit provides a rapid and easy method for the isolation of total RNA from formalin-fiex, paraffin-embedded (FFPE) tissue sections. Due to fixation and embedding procedures, nucleic acids in PPFE samples are heavily fragmented and sometimes modified by formaldehyde. While Mag-Bind FFPE RNA Kit is optimized to minimize the effect of the formaldehyde modification, it is not recommanded to use the RNA purified this kit for downstream applications that requires full length RNA.

Principle

The Mag-Bind FFPE RNA Kit combines high efficient binding properties of Mag-Bind technology with a specially designed buffer system to isolate total RNA sample from FFPE sample. Samples are first melt and lysed in RML Buffer with Protinase K. The lsate is them mixed with TRK Buffer and magnetic particles to bind the RNA on the surface of Mag-Bind particles. Genomic DNA is removed by DNase I digestion. After two wash steps, purified RNA is eluted with RNase-free water..

Storage and Stability

Most components in the Mag-Bind® FFPE RNA Kit should be stored at room temperature . Once dissolved, the Proteinase K and DNase I solution should be stored at -20°C. The Mag-Bind particles should be stored at 4-8°C for long term storage. with Store During shipping and storage, crystals may form in the TRK 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	M2551-00	M2551-01	M2551-02
Purification	1 x 96	4 x 96	20 x 96
Mag-Bind Particles R	1.1 ml	4.2 ml	21 ml
RML Buffer	35 ml	140 ml	700 ml
TRK Buffer	35 ml	140 ml	700 ml
GFC Buffer	10 ml	40 ml	200 ml
RWB Buffer	20 ml	100 ml	3 x 200 ml
DNase I digestion Buffer	10 ml	40 ml	200 ml
Proteinase K	40 mg	160 mg	800 mg
DEPC-ddH_2O	20 ml	40 ml	160 ml
Instruction Manual	1	1	1

* DNase I and DNase I digestion Buffer are shipped separately by dry ice.

Materials and Equipments supplied by user

- 96-100% ethanol (Do not use denatured alcohol)
- Nucelase-Free 96 deep-well plate (1.2 or 2.2 ml)
- Centrifuge with swing-bucket rotor capable of 14000 x g
- Sealing film
- RNase-free filter pipette tips
- Magnetic Separation device (MSD-01)
- Water bath or heat block Capable of 72°C
- Water bath or heat block Capable of 60°C
- Water bath or heat block Capable of 80°C
- Water bath or heat block Capable of 37°C

Before Starting

IMPORTANT	
	1. RWB Buffer must be diluted with absolute ethanol before use and store the diluted RNA wash buffer II at room temperature. M2251-00 Add 80 ml ~96-100% ethanol M2251-01 Add 400 ml ~96-100 % ethanol M2251-02 Add 800 ml ~96-100% ethanol 2. Proteinase K should be dissolved using DEPC Water and store the dissolved Proteinase K at -20°C.
	M2251-00 Add 2 ml water into each vial M2251-01 Add 8 ml water into each vial M2251-02 Add 40 ml water into each vial

Important Notes

Please take a few minutes to read this booklet thoroughly and become familiar with the protocol. Prepare all materials required before starting to minimize RNA degradation.

- Whenever working with RNA, always wear latex gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents.
- During the procedure work carefully but quickly.
- Under cool ambient conditions, crystals may form in TRK Buffer. This is normal and the bottle should be warmed to redissolve the salt.
- All centrifugation steps must be carried out at 22ºC-25ºC.

Note: Equilibrate samples and GFC Lysis buffer to room temperature before start. All steps must be carried out at room temperature. Work quickly, but carefully.

Starting Materials

Since standard formalin fixation and paraffin-embedding procedures always cause significant fragmentation of nucleic acids. We recommend following guideline 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 . Depend the yield and purity obtained, it may be possible to increase the starting material.

Mag-Bind FFPE RNA Protocol

1. **Cut the Paraffin sample sections 5-10 μM thick. Note: Do not use first 2-3 sections.**

2. **Immediately place the 3-8 sections into wells of a nuclease-free 96 deep-well plate contains 250μl RML Buffer.**

3. **Centrifuge at 4000 x g for 5 minutes.**

4. **Incubate at 72°C for 10 minutes to melt the paraffin. Mix the sample few times by gently shaking the plate 2-3 times. Make sure that the tissue sections stay submerged in the solution.**

5. **Add 20 μl Proteinase K to each well. Incubate at 55°C for 15-30 minutes with occasional mixing.**

6. **Incubate at 80°C for 10 minutes.**

7. **Centrifuge at 4000 x g at room temperature for 5 minutes. The paraffin will form a thin layer on top of the lysate solution.**

8. **Use a 1 ml pipette tip or large orifice tip to penetrate the paraffin layer, transfer 200 μl of lysate into a new 96 deep well plate.**

9. **Add 200 μl TRK Buffer and mix throughly by vortexing or pipetting up and down 10-20 times.**

10. **Add 20 μl Mag-Bind Particle R and 400 μl isopropanol to each sample. Mix throughly by vortexing or pipetting up and down 10-20 times. Incubate at room temperature for 5-10 minutes.**

11. **Collect magnetic particles: The total sample volume from step 9 will be around 850μl. If using MSD-01 magnet stand, a 500 μl Process Plate (EZ960-01/02) should be required for the rest of the protocol. If using magnet process robot such as KingFisger 96 instrument, please transfer entire sample into a KingFisher DW Plate and collected the magnetic particles, proceed to step 16.**

12. **Transfer 410 μl sample into a new 500 μl 96-well Process Plate (not supplied). Place the plate onto magnet separation device (MSD-01) to magnetize the magnetic particles. Wait 7-10 minutes or until all the magnetic particles are cleared from the solution.**

13. **Aspirate and discard the cleared supernatant.**

14. **Transfer remaining of the sample from step 10 (425 μl) into the 500μl process plate. Place the plate onto magnet separation device (MSD-01) to magnetize the magnetic particles. Wait 7-10 minutes or until all the magnetic particles are cleared from the solution.**

15. **Aspirate and discard the cleared supernatant.**

16. **Remove the plate from the magnetic separation device. Add 300 μl RWB Wash Buffer into each well. Resuspend the Mag-Bind Particles by pipetting up and down 20 times.**

17. **Place the plate onto magnet separation device (MSD-01) to magnetize the magnetic particles. Wait 7-10 minutes or until all the magnetic particles are cleared from the solution.**

18. **Aspirate and discard the cleared supernatant. Remove any liquid drop from each well with pipette.**

19. **Add 73.5 μl DNase I digest Buffer and 1.5 μl Rnase-free DNase I into each well. Resuspe e the plate are , to Centrifuge at Mix throughly by vortexing for 20 seconds.**

20. **Incubate at 37C for 15 minutes.**

21. **Add 75 μl GFC Buffer followed by adding 150 μl of isopropanol. Mix throughly by pipetting up and down for 20 times. Incubate at room temperature for 10 minutes.**

22. **Place the plate onto magnet separation device (MSD-01) to magnetize the magnetic particles. Wait 7-10 minutes or until all the magnetic particles are cleared from the solution.**

23. **Aspirate and discard the cleared supernatant.**

24. **Remove the plate from the magnetic separation device. Add 300 μl RWB Wash Buffer into each well. Resuspend the Mag-Bind Particles by pipetting up and down 20 times.**

25. **Place the plate onto magnet separation device (MSD-01) to magnetize the magnetic particles. Aspirate and discard the cleared supernatant.**

26. **Wash the Mag-Bind Particles with another 300 μl RWB by repeating step 24-25.**

27. Aspirate and discard all the liquid. Air dry the Mag-Bind particles by leave the plate on the magnetic separation device for 10 minutes. Remove any liquid drop using a pipette.

28. Add 30-50 µl DEPC Water into each well. Resuspend the Mag-Bind particles by pipetting up and down for 30 times. Incubate at room temperature for 10 minutes.

29. Place the plate onto magnet separation device (MSD-01) to magnetize the magnetic particles

30. Transfer the cleared supernatant contains purified RNA into a new nuclease-free 96-well microplate. Store the purified RNA at -80°C.

Trouble Shooting Guide

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.
	RWB Buffer were not prepared correctly.	Prepare RWB Buffer by adding ethanol according to instruction
	Loss of magnetic beads during operation	Becareful not to remove the magnetic beads during the operation
No RNA eluted.	RWB Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare RWB Wash Buffer Concentrate as instructed on the label.
Problem with downstream application	Insufficient RNA was used	1. RNA in the sample is already degraded; do not freeze and thaw the sample more than once or store at room temperature for too long 2. Quantify the purified RNA accurately and use sufficient RNA.
Carryover of the magnetic beads in the elution	Carryover from the magnetic beads in the eluted RNA will not effect downstream applications.	To remove the carryover magnetic beads from eluted RNA, simply magnetize the magnetic beads and carefully transfer to a new tube or plate.