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Introduction

Mag-Bind® FFPE RNA Kit provides a rapid and easy method for the isolation of total RNA from formalin-fixed, paraffin-embedded (FFPE) tissue sections using KingFisger 96 instrument. Up to 96 samples can be parallel processed on KingFisher 96 instrument. Due to fixation and embedding procedures, nucleic acids in FFPE samples are heavily fragmented and modified by formaldehyde. While the Mag-Bind FFPE RNA Kit is optimized to minimize the effect of the formaldehyde modification, it is not recommended to use the RNA purified with this kit for downstream applications that requires full length RNA.

Principle

The Mag-Bind FFPE RNA KF Kit is designed to isolate RNA from Formalin-fixed, paraffin-embedded tissue sample. This 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 treated with xylene to remove paraffin and lysed with RML Buffer with Proteinase K. The lysate is then mixed with MFB 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 KF 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. During shipping and storage, crystals may form in the MFB 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	M6593-00	M6593-01	M6593-02
Purification	1 x 96	4 x 96	20 x 96
Mag-Bind Particles R	2.2 ml	8.4 ml	42 ml
RML Buffer	15 ml	60 ml	300 ml
MFB Buffer	20 ml	80 ml	400 ml
GFC Buffer	32 ml	120 ml	2 x 300 ml
LPA Buffer	1.1 mL	4.4 mL	21 mL
RWB Buffer	25 ml	100 ml	3 x 200 ml
DNase I digestion Buffer	10 ml	40 ml	200 ml
DNase I	200 µl	4 x 200 µ	20 x 200 µ
Proteinase K	60 mg	240 mg	5 x 240 mg
Proteinase Storage Buffer	3ml	11 ml	55 ml
DEPC-ddH$_2$O	20 ml	40 ml	160 ml
Instruction Manual	1	1	1

Before Starting

Important

1. **RWB** Buffer must be diluted with Absolute ethanol before use and then store the diluted RWB Buffer at room temperature

M6593-00	Add 100 ml absolute ethanol
M6593-01	Add 400 ml absolute ethanol
M6593-02	Add 800 ml absolute ethanol

2. **GFC Buffer** must be diluted with isopropanol before use and then store the diluted GFC Buffer at room temperature

M6593-00	Add 48 ml Isopropanol
M6593-01	Add 180 ml Isopropanol
M6593-02	Add 450 ml Isopropanol

3. Proteinase K should be dissolved using Protease Storage Buffer and store the dissolved Proteinase K at -20°C.

M6593-00	Add 2.4 ml Protease Storage Buffer
M6593-01	Add 9.6 ml Protease Storage Buffer
M6593-02	Add 9.6 ml Protease Storage Buffer

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 37 °C to redissolve the salt.
- All centrifugation steps must be carried out at 22°C-25°C.

Note: Equilibrate samples and GFC Lysis buffer to room temperature before starting. All steps must be carried out at room temperature.

Starting Materials

Since standard formalin fixation and paraffin-embedding procedures 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 . Depending on the yield and purity obtained, it may be possible to increase the starting material.

Mag-Bind® FFPE RNA KF Protocol

Materials and Equipments supplied by User

- 96-100% ethanol (Do not use denatured alcohol)
- Nucelase-Free 96 round-well plate
- Centrifuge with swing-bucket rotor capable of 4000 x g
- Sealing film
- RNase-free filter pipette tips
- Magnetic Separation device (MSD-01B or MSD-01)
- Water bath or heat block Capable of 70°C
- Water bath or heat block Capable of 50°C
- Water bath or heat block Capable of 85°C
- Isopropanol
- Xylene

1. **Cut 3-6 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 to be used**.

3. **Immediately place the 3-5 sections into the wells of round well plate containing 1 ml xylene.**

4. **Mix throughly by vortexing for 20 seconds**.

5. **Incubate at 70°C for 3 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 the evaporation during incubation

6. **Centrifuge at 4000 x g at room temperature for 5 minutes to pellet the tissue.**

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

7. **Carefully remove the xylene without disturb the pellet. Discard the xylene.**

8. **Add 1 ml absolute ethanol (100%) to each well Mix throughly by vorexing for 20 seconds**.

9. Centrifuge at 4000 x g for 5 minutes to pellet the tissue sample. The pellet should be appear opaque.

10. **Carefully remove and discard the ethanol**. Add another 1 ml absolute ethanol to each sample. Resuspend pellet by vortexing for 20 seconds.

11. **Centrifuge at 4000 x g for 5 minutes at room temperature.**

12. **Carefully remove and discard the ethanol**. Remove any liquid drop with pipettor.

13. **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.

14. Add 140 μl RML Buffer and 20 μl proteinase K solution to each sample. Vortex the plate for 30 second to resuspend the pellet.

15. Incubate the plate at 50°C for 15 minutes and then 85°C for 10 minutes.

16. Centrifuge at 4000 x g for 5 minutes at room temperature.

17. Carefully transfer the cleared supernatant into a new 2 ml Kingfisher deep well (DW) plate.

18. Add 150 μl MFB Buffer followed by adding 20 μl Mag-Bind Particles RF and 300 μl to each well of the Kingfisher DW plate.

19. Import the Mag-Bind FFPE RNA Protocol into KingFisher 96 instrument.

20. Setup the KingFisher 96 instrument as following table:

Plate Type	Plate	Content	Reagent Volume
A	1	Lysate	150 µl
		MFB Buffer	150 µl
		Mag-Bind Particles RF	20 µl
		LPA Buffer(for small fragments)	10 µl
		Isopropanol	330 µl
A	2	RWB Buffer	600 µl
A	3	DNase I digestion mix (98 µl Digestion Buffer + 2 µl DNase I)	100 µl
		GFC Buffer (Added after the incubation)	600 µl
A	4	RWB Buffer	600 µl
B	5	DEPC Water	100 µl

* A= KingFisher 96 DW Plate, B=KingFisher 96 KF Plate.

21. Mix sample throughly on KingFisher 96 instrument (**plate 1**). RNA is bound to surface of the magnetic beads. Magnetic beads/RNA complex is collected by magnet on KingFisher 96 instrument.

22. Magnetic beads/RNA complex is washed with RWB Buffer in **plate 2.**

23. Magnetic beads/RNA complex is treated with DNase I mix in **plate 3.**

24. RNA is re-bound to magnetic beads in **plate 3** after adding MRW Buffer**.**

25. Magnetic beads/RNA complex is washed with RWB Buffer in **plate 4.**

26. RNA is eluted in **Plate 5.**

Trouble Shooting Guide

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 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	Increase the beads collection time
No RNA Eluted	GFC Buffer Concentrate not diluted with Isopropanol	Prepare GFC Buffer as instructed on the label**.**
	RWB Buffer were not prepared correctly	Prepare RWB Buffer by adding ethanol according to instruction
Problem with downstream application	Degraded RNA	Do not incubate sample over 15 minutes at step 15.
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.