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Introduction

The Mag-Bind™ Total RNA Kit is designed for rapid and reliable isolation of total RNA from cultured cells and soft animal tissues. By using innovative Mag-Bind™ RNA technology, Mag-Bind Total RNA kit provide uses a unique process for high quality RNA purification. Purified RNA is suitable for most downstream applications such as amplifications and enzymatic reactions. The procedure can be scaled up or down, allowing for the purification from various amounts of starting materials.

Principle

If using the Mag-Bind™ Total RNA Kit for the first time, please read this booklet to become familiar with the procedure and its various modifications. Samples are lysed in a specially formulated buffer containing detergent and proteinase K. RNA was bound to the surface of Mag-Bind magnetic particles under proper condition. The magnetic particles are separated from lysates using a magnet. Proteins and cellular debris are efficiently washed away. Next, the nucleic acid is treated with DNase, and purified from the reaction mixture using a second magnetic beads binding and washing procedure. Purified RNA is then eluted in nuclease-free water or low ionic strength buffer.

Storage and Stability

The Mag-Bind™ Total RNA Kit are stable for at least 12 months from date of purchase. During shipment, or storage in cool ambient conditions, precipitates may form in the MCL-1 Lysis Buffer. It is possible to dissolve such deposits by warming the solution at 37°C, though we have found that they do not interfere with overall performance. DNase I, DNase Digestion Buffer has to be stored at -20°C. Mag-Bind Particle R need to be stored at 4-8° C.

Kit Contents

Product	M6930-00	M6930-01	M6930-02
Purification	5	50	200
Mag-Bind™ Particles Solution R *	110 µl	1.1 ml	4.4 mL
MCR-1 Buffer	2 mL	20 mL	80 mL
MCR-2 Buffer	300 µl	3 mL	12 mL
MBW Buffer	8 ml	80 ml	320 ml
SPR Wash Buffer	5 mL	16 mL	50 mL
Proteinase K	3 mg	30 mg	4 x 30 mg
Proteinase Storage Buffer	160 µl	3 ml	12 ml
DEPC-Water	1.2 mL	10 mL	40 mL
DNase I**	9 µl	88 µl	355 µl
DNase Digestion Buffer**	1 mL	7 mL	30 mL
User Manual	1	1	1

Product	M6731-00	M6731-01	M6731-02
Purification	1 x 96	4 x 96	12 x 96
Mag-Bind™ Particles R *	2.15 ml	8.6 ml	25.8 ml
MCR-1 Buffer	20 ml	80 mL	240 mL
MCR-2 Buffer	2.5 ml	10 mL	40 mL
MBW Buffer	60 ml	250 ml	750 ml
SPR Wash Buffer	25 mL	100 mL	3 x 100 mL
Proteinase K	30 mg	120 mg	3 x 120 mg
Proteinase Storage Buffer	2 ml	6 ml	18 ml
DEPC-Water	15 mL	60 mL	180 mL
DNase I**	160 µl	480 µl	1440 µl
DNase Digestion Buffer**	5.5 mL	22 mL	70 mL
User Manual	1	1	1

* Store Mag-Bind Particle solution R at 4-8°C
**DNase I and DNase digestion Buffer has to be shipped and stored at -20C.

Before Starting

IMPORTANT		
	1. SPR Wash Buffer must be diluted with absolute ethanol before use.	
	M6930-00	Add 20 ml absolute ethanol (96-100%)
	M6930-01	Add 84 ml absolute ethanol (96-100%)
	M6930-02	Add 200 ml absolute ethanol (96-100%)
	M6731-00	Add 100 ml absolute ethanol (96-100%)
	M6731-01	Add 400 ml absolute ethanol (96-100%)
	M6731-02	Add 400 ml absolute ethanol (96-100%)
	2. Dissolve Proteinase K with DEPC Water and store at -20°C.	
	M6930-00	Add 150 µl Protease Storage Buffer to the vial
	M6930-01	Add 1.5 ml Protease Storage Buffer to the vial
	M6930-02	Add 1.5 ml Protease Storage Buffer to the vial
	M6731-00	Add 1.2 ml Protease Storage Buffer to the vial
	M6731-01	Add 4.8 ml Protease Storage Buffer to the vial
	M6731-02	Add 4.8 ml Protease Storage Buffer to the vial

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

- 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.

Starting Material

The Mag-Bind™ Total RNA Kit is optimized for RNA purification from up to 5 x 10^6 cells and 5-10 mg animal or human tissue sample by using single tube protocol. For 96-well plate format, due to the volume limitation of the microplate, up to 2.5 x 10^6 cells and 2.5-5 mg animal or human tissue sample should be used. However, for KingFisher instrument, larger starting sample can be processed by increasing the starting volume of sample and all the reagents proportionally.

Amount of starting material and elution volumes used for Mag-Bind™ Total RNA isolation procedure.

Sample	Amount of Starting material	Elution Volume
Cultured cells	≤ 5 x 10^6 cells	50-100 µL
White Blood Cells	≤ 5 x 10^6 cells	50-100 µL
Bacterial	≤ 5 x 10^7 cells	50-100 µL
Liver or spleen	5 mg	50-100 µL

Typical Total RNA Yield

RNA yield varies depending on the type of the cell line and storage condition. The average RNA yield using The Mag-Bind™ Total RNA Kit is 5-20 pg per cell.

Typical Total RNA Yield Using Mag-Bind™ Total RNA Kit

Sample	RNA yield (µg per 10^6 Cells)
NIH/3T3	10 µg
Huh	10-30 µg
Lung	3-16 µg
Heart	3-20 µg
Brain	4-24 µg
Adipose tissue	3-16 µg

Mag-Bind™ Total RNA Isolation Using Microcentrifuge Tube

Material and Equipments supplied by User

Have the following reagents and supplies ready before starting procedure:

- Magnetic separation device for 1.5 ml/2ml Tube (MSD-02)
- Nuclease-free 1.5 mL centrifuge tube
- Table top centrifuge capable at least 13,000 x g
- Nuclease-Free centrifuge tubes.
- Absolute ethanol (96-100%)
- Isopropanol

Protocol

Prepare lysis master mix as following table and use within 10 minutes – discard any unused solution.

Number of sample	MCR-1	MCR-2	Proteinase K	Total volume
1	360 µl	40 µl	10 µl	410 µl
2	792 µl	88 µl	22 µl	902 µl
3	1188 µl	132 µl	33 µl	1353 µl
4	1584 µl	176 µl	44 µl	1804 µl
10	3.96 ml	440 µl	110 µl	4.5 ml

Note: calculated volume represent 10% extra to compensate the lose of the volume during pipetting.

1. Disrupt and lysis of samples, choose a method below:

A) Cells Grown in Suspension

Pellet cells by centrifugation in a centrifuge tube. Lyse cells in 410 ul lysis master mix by repetitive pipetting. Alternately, vortex the tube at maximum speed for 30 seconds to lysis the cell. Use 410 µL of the lysis master mix per 5 x 10^6 of animal, yeast cells, or per 5 x 10^7 bacterial cells. Washing cells before addition of lysis master mix should be avoided as this increases the possibility of mRNA degradation and RNase contamination

B) Cells Grown in Monolayer

Lyse cells directly in a culture plate or dish by adding 410 µL of lysis master mix to a each well of multiwell cell culture plate, and passing the cell lysate several times through a blue pipette tip. Alternately, vortex the tube at maximum speed for 30 seconds to lysis the cell. The amount of lysis master mix added is based on the area of the culture plate (~400 µL per 2 cm^2). An insufficient amount of lysis master mix may result in contamination of the isolated RNA with DNA.

2. **Incubate the sample at 55°C for 10 minutes.**

3. **Add 20 µl Mag-Bind™ Particles Solution R and add equal volume of Isopropanol(430 ul).** Mix throughly by vortexing 30 seconds.

   Note: Vortex the Mag-Bind™ Particles Solution R at moderate speed to form a uniform suspension before pipetting. For multiple samples, a master mix of Isopropanol and **Mag-Bind™** Particles solution R can be prepared.

4. Incubate at room temperature for 10 minutes.

5. **Place the tube on a magnetic separation device to magnetize the magnetic particles.** Leave the tube on the magnet until all the magnetic particles are pelleted.

6. **Carefully remove the cleared supernatant by using a pipettor without disturbing the magnetic particles.**

7. **Remove the tube containing the magnetic particles from the magnetic separation device**. Add 600 µl of MBW Buffer and resuspend magnetic particles pellet by vortexing the tube maximum speed for 30 seconds**.**

8. **Place the tube onto a magnetic separation device to magnetize the magnetic particles**. Aspirate the cleared supernatant after the lysate is cleared.

9. **Remove the Tube containing the magnetic particles from the magnetic separation device**. Add 600 µl of SPR Wash Buffer to each well and resuspend magnetic particles pellet vortexing for 30 seconds.

10. **Place the tube onto a magnetic separation device to magnetize the magnetic particles. Aspirate the cleared supernatant after the lysate is cleared. Air dry for 5 minutes.**
    Note: It is very important to remove any liquid drop from the wells of the process plate before adding the DNase I digestion mix.

11. **Leave the tube on the magnetic separation device. Prepare the DNase I digestion mix as following:**

Number of Samples	DNase I Digestion Buffer	DNase I	Total Volume
1	98.5 µl	1.5 µl	100 µl
2	217 µl	3 µl	220 µl
3	325 µl	4.5 µl	329.5 µl
4	434 µl	6 µl	440 µl
10	1084 µl	16.5 µl	1.1 ml

12. **Add 100 µl of DNase I digestion Buffer and resuspend the magnetic particles by pipetting up and down for 20 times**. Incubate at room temperature for 10-15 minutes**.**

13. **Add 550 µl MBW Buffer to each sample and mix throughly by pipetting up and down for 20 times.** Incubate at room temperature for 10 minutes.

14. **Place the tube onto a magnetic separation device to magnetize the magnetic particles.** Aspirate the cleared supernatant after the lysate is cleared.

15. **Add 600 µl of SPR Wash Buffer and resuspend magnetic particles pellet by vortexing at maximum speed for 30 seconds.**

16. **Place the tube onto a magnetic separation device to magnetize the Mag-Bind™ particles.**

17. **Aspirate and discard the cleared supernatant**. Carefully remove any liquid drop from tube. Leave the tube on the magnet stand. Air dry the magnetic particles at room temperature for 7-10 minutes.

18. **Add 50 µl DEPC Treated water and resuspend magnetic particles pellet by vortexing or pipetting up and down for 20 times**. Incubate at room temperature for 3 minutes.

19. **Place the tube onto a magnetic separation device to magnetize the Mag-Bind™ particles**.

20. **Transfer the cleared supernatant contains purified RNA into a new 1.5 ml tube**. Store the purified RNA at -80°C.

Mag-Bind™ Total RNA Isolation Using 96-well Plate (M6731)

Material and Equipments supplied by User

Have the following reagents and supplies ready before starting procedure:

- Magnetic separation device for 96-well plate (MSD-01)
- Nuclease-free microplate
- Nuclease-free filter tips
- Multichannel pipett
- Centrifuge capable of at least 4,000 x g
- Swinging -bucket rotor and adapter for 96-well plate
- Absolute ethanol (96-100%)
- Isopropanol

Protocol

Prepare lysis master mix as following table and use within 10 minutes – discard any unused solution.

Number of Sample	MCR-1	MCR-2	Proteinase K	Total volume
1	180 µl	20 µl	10 µl	210 µl
2	396 µl	44 µl	22 µl	462 µl
3	594 µl	66 µl	33 µl	630 µl
4	792 µl	88 µl	44 µl	924 µl
8	1. 6 ml	180 µl	88 µl	1.87 ml
16	3.2 ml	360 µl	176 µl	3.74 ml
24	4.8 ml	540 µl	270 µl	5.6 ml
48	9.6 ml	1.08 ml	540 µl	11.2 ml
96	19.2 ml	2.16 ml	1060 µl	22.4 ml

Note: calculated volume represent 10% extra to compensate the lose of the volume during pipetting.

1. **Disrupt and lysis of samples, choose a method below:**

   A) Cells Grown in Suspension
   Pellet cells by centrifugation. Lyse cells by adding 210 ul lysis master mix into each well of the plate. Mix throughly by repetitive pipetting. Alternately, vortex the plate at maximum speed for 30 seconds to lysis the cell. Use 210 µL of the lysis master mix per 2.5 x 10^6 of animal, yeast cells, or per 2.5 x 10^7 bacterial cells. Washing cells before addition of lysis master mix should be avoided as this increases the possibility of mRNA degradation and RNase contamination

   B) Cells Grown in Monolayer
   Lyse cells directly in a culture plate or dish by adding 210 µL of lysis master mix to a each well of multiwell cell culture plate, lyse and homogenize the sample by pipetting up and down 20 times. Alternately, vortex the plate at maximum speed for 30 seconds to lysis the cell.

   The amount of lysis master mix added is based on the area of the culture plate (~200 µL per 1 cm^2). An insufficient amount of lysis master mix may result in contamination of the isolated RNA with DNA.

2. **Incubate the sample at 55°C for 10 minutes.**

3. **Add 20 µl Mag-Bind™ Particles Solution R and add equal volume of Isopropanol(230 ul).** Mix throughly by vortexing 30 seconds.

   Note: Vortex the Mag-Bind™ Particles Solution R at moderate speed to form a uniform suspension before pipetting. For multiple samples, a master mix of Isopropanol and **Mag-Bind™** Particles solution R can be prepared.

4. Incubate at room temperature for 10 minutes.

5. **Place the plate on a magnetic separation device to magnetize the magnetic particles.** Leave the plate on the magnet until all the magnetic particles are pelleted.

6. **Carefully remove the cleared supernatant by using a pipettor without disturbing the magnetic particles.**

7. **Remove the tube containing the magnetic particles from the magnetic separation device**. Add 300 µl of MBW Wash Buffer and resuspend magnetic particles pellet by pipetting up and down 20 times**.**

8. **Place the plate onto a magnetic separation device to magnetize the magnetic particles**. Aspirate the cleared supernatant after the lysate is cleared.

9. **Remove the plate from the magnetic separation device**. Add 350 µl of SPR Wash Buffer to each well and resuspend magnetic particles pellet by pipetting up and down for 20 times.

10. **Place the plate onto a magnetic separation device to magnetize the magnetic particles. Aspirate the cleared supernatant after the lysate is cleared. Air dry the beads for 5 minutes.**

    Note: It is very important to remove any liquid drop from the wells of the process plate before adding the DNase I digestion mix.

11. **Leave the tube on the magnetic separation device. Prepare the DNase I digestion mix as following:**

Number of Samples	DNase I Digestion Buffer	DNase I	Total Volume
8	427 µl	13 µl	440 µl
16	854 µl	26 µl	880 ml
24	1.28 ml	40 µl	1.32 ml
48	2.6 ml	85 µl	2.68 ml
96	5.2 ml	170 µl	5.37 ml

12. **Add 50 µl of DNase I digestion Buffer to each well of sample and resuspend the magnetic particles by pipetting up and down for 20 times**. Incubate at room temperature for 10-15 minutes**.**

13. **Add 275 µl MBW Buffer to each sample and mix throughly by pipetting up and down for 20 times.** Incubate at room temperature for 10 minutes.

14. **Place the plate onto a magnetic separation device to magnetize the magnetic particles.** Aspirate the cleared supernatant after the lysate is cleared.

15. **Add 300 µl of SPR Wash Buffer and resuspend magnetic particles pellet by pipetting up and down 20 times.**

16. **Place the plate onto a magnetic separation device to magnetize the Mag-Bind™ particles.**

17. **Aspirate and discard the cleared supernatant**. Carefully remove any liquid drop from each well. Leave the plate on the magnet stand. Air dry the magnetic particles at room temperature for 7-10 minutes.

18. **Add 50 µl DEPC Treated water and resuspend magnetic particles pellet by vortexing or pipetting up and down for 20 times**. Incubate at room temperature for 3 minutes.

19. **Place the plate onto a magnetic separation device to magnetize the Mag-Bind™ particles**.

20. **Transfer the cleared supernatant contains purified RNA into a new microplate**. Seal the plate with sealing film and store the purified RNA at -80°C.

Trouble Shooting

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.
	SPR Buffer were not prepared correctly.	Prepare the SPR Buffer by adding ethanol according to instruction
	Loss of magnetic beads during operation	Becareful not to remove the magnetic beads during the operation
	Undissolved particles in the cell lysate cause congregation of magnetic beads	make sure the lysate is clear of particles before adding magnetic beads.
No RNA eluted.	VRB and SPR Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare VRB and SPR 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.
DNA contamination	Inefficient of DNase I digestion	1. Make sure to use proper starting material 2. Ensure that the temperature of DNase I digestion at 20-25° C