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Introduction

The Mag-Bind® Total RNA Kit is designed for rapid and reliable isolation of Total RNA from cultured cells. The innovative Mag-Bind® RNA technology provide high quality RNA, which is suitable for direct use in most downstream applications such as amplifications and enzymatic reactions. This system can be easily adapted with an automated system and 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. Finally, purified RNA is then eluted in nuclease-free water or low ionic strength buffer. Purified RNA can be directly used in downstream applications without the need for further purification.

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 TRK 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. Magsi RNA Particle and RNA-Solv Reagent need to be stored at 2-8° C. Proteinase K can be stored at 15-25 °C.

Kit Contents

Product	M6930-00	M6930-01	M6930-02
Purification	5	50	200
MagSi RNA Particles	110 µl	1500 µl	5 ml
TRK Lysis Buffer	5 ml	30 ml	100 ml
RNA-Solv Reagent	5 ml	50 ml	200 ml
MBW Wash Buffer (Buffer P3)	4.4 ml	22 ml	88 ml
SPR Wash Buffer	5 ml	20 ml	2 x 50 ml
Proteinase K	120 µl	1.2 ml	4 .8 ml
DEPC-Water	1 ml	10 ml	30 ml
DNase I **	8 µl	80 µl	4 x 80 µl
10 x DNase Digestion Buffer**	200 ul	1 ml	2 x 1.5 ml
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* Store MagSi RNA Particle at 2-8°C

**DNase I and DNase digestion Buffer has to be shipped and stored at -20°C.

Before Starting

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*.

IMPORTANT	**1. SPR Wash Buffer** must be diluted with absolute ethanol before use.	
	M6930-00	Add 20 ml 100% ethanol
	M6930-01	Add 80 ml 100% ethanol
	M6930-02	Add 200 ml 100% ethanol
	2. MBW Wash Buffe Must be diluted with absolute ethanol before use.	
	M6930-00	Add 5.6 mL absolute ethanol
	M6930-01	Add 28 ml absolute ethanol
	M6930-02	Add 112 ml absolute ethanol

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%)
- Isopropano

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.

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
Tissues	<10 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
Muscle	3-15 µg
Adipose tissue	3-16 µg

Mag-Bind® Total RNA Standard Protocol

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 400 ul TRK Lysis Buffer by repetitive pipetting. Alternately, vortex the tube on a at maximum speed for 30 seconds to lysis the cell. Use 400 µL of the reagent per 5 x 10^6 of animal, yeast cells, or per 5 x 10^7 bacterial cells. Washing cells before addition of Lysis Buffer 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 400 µL of TRK Lysis Byffer 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 Buffer added is based on the area of the culture plate (~400 µL per 2 cm^2). An insufficient amount of Lysis Buffer Buffer may result in contamination of the isolated RNA with DNA.

   C) Tissue Samples
   Lyse 5-10 mg tissues with 400 µL TRK Lysis Buffer. Tissue samples can be disrupt or homogenize by Liquid Nitrogen or rotor stator. For liver or spleen, do not used >5 mg.

   Note: Add 20 µl 2-mercaptoethanol per 1 ml of TRK Lysis Buffer before use. This mixture can be made and stored at room temperature for 1 week.

2. **Add 300µl absolute ethanol and 20 µl Proteinase K to the sample.** Shaking to mix for 1 minutes.

3. **Add 20 µl MagSi magnetic Particles and** shaking for 5 minutes.
   Note: Vortex the magnetic Beads at moderate speed to form a uniform suspension before pipetting.

4. **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.

5. **Carefully aspirate and discard the cleared supernatant without disturbing the magnetic particles.**

6. Remove the tube containing the magnetic particles from the magnetic separation device. **Add 600 µl of MBW Wash Buffer and resuspend magnetic particles pellet by shaking the tube at maximum speed for 1 minutes.**

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

8. Remove the Tube containing the magnetic particles from the magnetic separation device. **Add another 600 µl of SPR Wash Buffer to each well and resuspend magnetic particles pellet shaking for 1 minutes.**

9. 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 10 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.

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

Number of Samples	10 x DNase Digestion Buffer	DEPC-Water	DNase I	Total Volume
1	10 µl	88.5 µl	1.5 µl	100 µl

11. **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.**

12. **Add 400 µl SPR Wash Buffer to each sample** and mix throughly by shaking for 5 minutes.

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

14. **Add 500 µl of SPR Wash Buffer and resuspend magnetic particles pellet by vortexing at maximum speed for 30 seconds.**

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

16. **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.

17. **Add 50-100 µl DEPC Treated water and resuspend magnetic particles pellet by vortexing or or pipetting up and down for 20 times**. Incubate at room temperature for 3 minutes.

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

19. Transfer the cleared supernatant contains purified RNA into a new 1.5 ml tube.

20. Store the purified RNA at -80°C.

Mag-Bind® Total RNA Universal Protocol

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 750 ul RNA-Solv Reagent by repetitive pipetting. Alternately, vortex the tube on a at maximum speed for 30 seconds to lysis the cell. Use 700 µL of the reagent per 5 x 10^6 of animal, yeast cells, or per 5 x 10^7 bacterial cells. Washing cells before addition of Lysis Buffer 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 750 µL of RNA-Solv Reagent 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 Buffer added is based on the area of the culture plate (~750 µL per 2 cm^2). An insufficient amount of Lysis Buffer may result in contamination of the isolated RNA with DNA.

C) Tissue Samples

Lyse 10-50 mg tissues with 750 µL RNA-Solv Reagent. Tissue samples can be disrupt or homogenize by Liquid Nitrogen or rotor stator. For liver or spleen, do not used >20 mg.

2. **Add 150 µl chloroform and cap sample tubes securely and shake vigorously for 15 seconds.** Incubate on ice for 10 minutes. *This step is critical - do not change it*.

3. Centrifuge the samples at no more than 12,000 x g for 15 minutes 4°C. The mixture separates into a lower phenol-chloroform phase, an interphase, and an upper aqueous phase. RNA remains entirely in the aqueous phase.

4. Transfer **300 µl of** the supernatant into a new tube.

5. Add 20 µl MagSi Particles and 220 µl of Isopropanol. Mix throughly by shaking for 5 minutes.

6. **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.

7. **Carefully aspirate and discard the cleared supernatant without disturbing the magnetic particles.**

8. Remove the tube containing the magnetic particles from the magnetic separation device. **Add 600 µl of MBW Wash Buffer and resuspend magnetic particles pellet by shaking the tube at maximum speed for 1 minutes.**

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

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

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

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

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

14. **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 10-15 minutes.

15. **Add 50-100 µl DEPC Treated water and resuspend magnetic particles pellet by vortexing or or pipetting up and down for 20 times**. Incubate at room temperature for 3 minutes.

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

17. Transfer the cleared supernatant contains purified RNA into a new 1.5 ml tube.

18. Store the purified RNA at -80°C.

Trouble Shooting Guide

Problem	Likely Cause	Suggestion
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