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Revised January 2009

Introduction

Mag-Bind™ Plant RNA Kit allows rapid and reliable isolation of high-quality total cellular RNA from a wide variety of plant species and tissues. Total RNA from 40 mg of sample can be processed in less than 1 hour. The system uses a innovative Mag-Bind plant RNA technology to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from plant lysate and provide high quality RNA. Purified RNA is suitable for all major downstream applications such as RT-PCR, restriction digestion, and hybridization techniques. Since this kit uses magnetic beads, the protocol can be easily adapted to most robotic liquid handling instruments.

Overview

If using the Mag-Bind™ Plant RNA Kit for the first time, please read this booklet in its entirety to become familiar with the procedures. Samples are lysed in a specially formulated buffer containing detergent. Nucleic aids (DNA/RNA) are captured by Mag-Bind particles and separated from proteins and other cell debris on a magnet. Genomic DNA is effectively removed with a quick DNase treatment. RNA is then re-bound to Mag-Bind particles and eluted with DEPC-treated water.

Storage and Stability

Most components of the Mag-Bind™ Plant RNA Kit are stable for at least 12 months from date of purchase when stored at 22ºC-25ºC. Mag-Bind® Particles Solution should be stored at 4°C. DNase I has to be stored at -20C. During shipment or storage in cool ambient conditions, precipitates may form in Buffer MRPL. Dissolve such deposits by warming the solution at 37ºC and gently shaking.

Kit Contents

Product Number	M6954-00	M6954-01	M6954-02
Purification	5	50	200
Mag-Bind® Particles Solution R	100 µL	1 mL	4 mL
Buffer MRPL	4 mL	40 mL	160 mL
Buffer SP2	1.3 mL	13 mL	55 mL
RWB	2 mL	12 mL	50 mL
MRW Wash Buffer	6 mL	60 mL	250 mL
Proteinase K	1.5 mg	15 mg	60 mg
DNase I Digestion Buffer	600 µL	6.0 mL	25 mL
DNase I	12 µL	120 µL	440 µL
DEPC Water	600 µL	10 mL	40 mL
Instruction Booklet	1	1	1

Product Number	M6927-00	M6927-01	M6927-02
Purification	1 x 96	4 x 96	12 x 96
Mag-Bind® Particles Solution R	1.1 mL	4.4 mL	12.8 mL
Buffer MRPL	30 mL	120 mL	360 mL
Buffer SP2	10 mL	40 mL	120 mL
RWB	20 mL	50 mL	150 mL
MRW Wash Buffer	60 mL	250 mL	750 mL
Proteinase K	30 mg	120 mg	360 mg
DNase I	100 µL	4 x 100 µL	12 x 100
DNase I Digestion Buffer	6 mL	25 mL	75 mL
DEPC Water	15 mL	60 mL	250 mL
Instruction Booklet	1	1	1

Before Starting

Please take a few minutes to read this manual thoroughly to become familiar with the protocol before beginning the procedure. Prepare all materials required before starting 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.

- Under cool ambient conditions, crystals may form in MRPL Buffer. This is normal and the bottle should be warmed and gently shaken to re-dissolve the deposits.

- The paramagnetic particles in Mag-Bind® Particles Solution R will bead together after a short while. The particles *must be* fully dispersed in the solution to obtain optimal yields. Vigorously shake or vortex the container before beginning the procedure to resuspend the particles. Depending on length of operation, the particles might need to be resuspened again
- 2-mercaptoethanol (ß-mercaptoethanol) may play important role in denaturing RNases and can be a optional addition to MRPL Buffer before use. Add 20 µl of 2-mercaptoethanol per 1 ml of MRPL Buffer. This mixture can be stored for one month at room temperature. If storage is beyond one month, 2-mercaptoethanol should be added again.

- Reconstitute Proteinase K with DEPC water as follows and **store at -20°C**

M6954-00	Add 60 µl DEPC water
M6954-01	Add 600 µl DEPC water
M6954-02	Add 2.4 ml DEPC water
M6927-00	Add 1.2 ml DEPC water
M6927-01	Add 4.8 ml DEPC water
M6927-02	Add 14.4 ml DEPC water

- Dilute RWB Buffer with absolute ethanol as follows and **store at room temperature**.

M6954-00	Add 8 ml absolute ethanol
M6954-01	Add 48 ml absolute ethanol
M6954-02	Add 200 ml absolute ethanol
M6927-00	Add 80 ml absolute ethanol
M6927-01	Add 200 ml absolute ethanol

M6927-02	Add 600 ml absolute ethanol

Mag-Bind™ Plant RNA Protocol using 1.5 ml tube (M6954)

Materials to be provided by user:

- Centrifuge capable of 12,000 x g.
- Nuclease-free 1.5 mL centrifuge tube.
- Absolute (96%-100%) ethanol
- Isopropanol
- Equipment for disrupting plant tissue (MM300 Mixer Mill or Geno/Grinder 2000)
- Mortar and pestle (for manual tissue disruption)
- Magnetic Stand for 1.5ml tube (MSD-02)

Tissue Disruption

Manual disruption:
To prepare samples, collect fresh plant sample in a 30 mL mortar and freeze by dipping in liquid nitrogen using tweezers or tongs to fill the tube. Grind the tissue using a clean pestle. Allow the liquid nitrogen to evaporate and transfer the sample into a 1.5 ml centrifuge tube. Immediately proceed with the RNA isolation protocol.

Mechanical tissue disruption:
Place sample into a stainless steel grinding jar with appropriate steel beads. Freeze samples in the stainless steel grinding jar using liquid nitrogen for 1 minute. Immediately attach the grinding jar onto the clamps of the Tissue lysate. Grind tissue at 30Hz for 1-2 minutes.

1. **Collect ground plant sample (start with 30-40 mg) in a 1.5 mL centrifuge tube**.

2. **Immediately add 600 µL Buffer MRPL and 10 µl Proteinase K solution (25 mg/ml)**. Mix the sample throughly by vortexing at maximum speed for 30-60 seconds.

3. **Add 200 µL Buffer SP2 and vortex to mix throughly**.

   Note: It is critical to mix the sample throughly by vortexing vigorously to give optimized yield.

4. **Incubate 10 minutes at -20°C**. This step helps to remove the proteins, polysacchrides and other inhibitors.

5. **Centrifuge at 14,000 x g for 10 min to pellet cell debris**. Compact pellets will form in the tubes but some particles may float. Be careful to avoid those particles during the transfer in next step.

6. **Carefully transfer 400 µl cleared supernatant to a new 1.5 mL microcentrifuge tube**. Making sure not to disturb the pellet or transfer any debris.

7. **Add 20µl Mag-Bind Particles Solution R follow by 420 µL of isopropanol**. Mix throughly by vortexing.

8. **Incubate at room temperature for 5 minutes.** Mix the sample few times during the incubation.

9. **Place the tube on a magnetic separation device suitable for 1.5 mL tube to magnetize the Mag-Bind® particles.**

10. **Aspirate and discard the cleared supernatant**. Do not disturb the magnetic beads.

11. Add 500 µL of MRW Buffer. **Resuspend Mag-Bind™ particles pellet by vortexing**. Incubate 3 minutes at room temperature.

12. **Place the tube onto a magnetic separation device to magnetize the Mag-Bind™particles**. Aspirate and discard the cleared supernatant.

13. Remove the tube containing the Mag-Bind® particles from the magnetic separation device. **Add 500 µL of RWB Wash Buffer (diluted with ethanol see page 4 for instruction) into the tube.**

14. **Resuspend Mag-Bind® particles pellet by vortexing the tube for 30 seconds.**

15. **Place the tube onto a magnetic separation device to magnetize the Mag-Bind® particles**. Remove and discard the cleared supernatant.

16. **Remove tube from magnet and add 98µl DNase I Digestion Buffer and 2 µl DNase I**. Resuspend the magnetic beads by pipetting or gent mix.

Note: If total nucleic acid (both DNA and RNA) are desired, skip the DNase I digestion step and proceed the wash step starting from step 20.

17. **Incubate at room temperature (22-25°C) for 10 minutes.**

18. **Add 550 µl MRW Buffer to the tube and mix throughly by vortexing for 20 seconds.** Incubate 5 minutes at room temperature.

19. **Place the tube on magnet stand to collect the magnetic beads.** Carefully remove and discard the cleared supernatant.

20. **Remove the tube from magnet and add 500 µl RWB Buffer**. Resuspend the magnetic beads by vortexing.

21. **Place the tube on magnet stand to collect the magnetic beads.** Carefully remove and discard the cleared supernatant

22. **Leave the tube on the magnet to air dry the magnetic beads for 5 minutes**. Remove any residue liquid from tube by pipetting.

23. Remove the tube from magnetic separation device. Add 50-100 ul DEPC-treated water and resuspend the beads by vortexing.

24. Incubate 5-10 minutes at room temperature.

25. Place the tube onto magnet to magnetize the Mag-Bind® particles.

26. Transfer the cleared supernatant containing purified RNA to a new 1.5 mL tube.

Mag-Bind™ Plant RNA Protocol using 96-well Plate (M6927)

Materials to be provided by user:

- Centrifuge capable of 4,000 x g with swinging-bucket rotor for 96 well plates
- Sealing Film
- 2 ml deep-well plate (Cat#EZ9602-02)
- 500 µl round well processing plate (Cat#EZ9604-02)
- Absolute (96%-100%) ethanol
- Isopropanol
- Equipment for disrupting plant tissue (MM300 Mixer Mill or Geno/Grinder 2000)
- Mortar and pestle (for manual tissue disruption)
- Magnetic Stand for 96-well plate (Cat# MSD-01)

To prepare samples, collect fresh plant sample in a 30 mL mortar and freeze by dipping in liquid nitrogen using tweezers or tongs to fill the tube. Grind the tissue using a clean pestle. Transfer the grounded powder and liquid nitrogen into 96 deep-well plate and allow the liquid nitrogen to evaporate. Immediately proceed with the RNA isolation protocol.

Mechanical tissue disruption:
Place sample into a stainless steel grinding plate with appropriate steel beads. Freeze samples in the stainless steel grinding jar using liquid nitrogen for 1 minute. Immediately attach the grinding jar onto the clamps of the Tissue lysate. Grind tissue at 30Hz for 1-2 minutes.

1. **Collect ground plant sample (start with 15-20 mg) in a deep well process plate.**

   Note: For **Kingfisher 96 instrument**, the starting material can be doubled to 30-40 mg. Double all the reagent volume in this protocol.

2. **Immediately add 300 µL Buffer MRPL and 10 µl Proteinase K solution (25mg/ml)**. Seal the plate with Sealing film and mix the sample throughly by vortexing at maximum speed for 30-60 seconds.

3. **Add 100 µL Buffer SP2 and** seal the plate with Sealing film **vortex to mix throughly**.

Note: It is critical to mix the sample throughly by vortexing vigorously to give optimized yield.

4. **Incubate 10 minutes at -20°C**. This step helps to remove the proteins, polysacchrides and other inhibitors.

5. **Centrifuge at 4,000 x g for 20 min to pellet cell debris**. Compact pellets will form in the plate but some particles may float. Be careful to avoid those particles while transferring the supernatant in next step.

6. **Carefully transfer 200 µl of cleared supernatant to a 500 µl processing plate**. Making sure not to disturb the pellet or transfer any debris.

7. **Add 10µl Mag-Bind Particles Solution R follow by 210 µL of isopropanol**. Mix by Pipetting up and down 10 times.

8. **Incubate at room temperature for 5 minutes.** Mix the sample 3 times during the incubation by pipetting.

9. **Place the plate on magnetic stand (Cat# MSD-01) suitable for 96-well microplate to magnetize the Mag-Bind® particles.**

10. Wait until the magnetic beads form a pellet in the side of the wells (if using MSD-01) or until the solution is clear(if using another magnet). **Aspirate and discard the cleared supernatant**. Do not disturb the magnetic beads.

11. Add 300 µL of MRW Buffer **Resuspend Mag-Bind™ particles pellet by pipetting up and down 10-20 times.** Incubate 3 minutes at room temperature.

12. **Wait until the magnetic beads form a pellet in the side of the wells (if using MSD-01) or until the solution is clear(if using another magnet). Place the plate onto a magnetic separation device to magnetize the Mag-Bind™particles**. Aspirate and discard the cleared supernatant.

13. Remove the plate containing the Mag-Bind® particles from the magnetic stand. **Add 300 µL of RWB Wash Buffer (diluted with ethanol see page 4 for instruction) into the plate.**

14. **Resuspend Mag-Bind® particles pellet by pipettng up and down 10-20 times.**

15. **Place the plate onto a magnetic separation device to magnetize the Mag-Bind® particles**. **Wait until the magnetic beads form a pellet in the side of the wells (if using MSD-01) or until the solution is clear(if using another magnet)** Remove and discard the cleared supernatant.

16. **Remove plate from magnet and add 48µl DNase I Digestion Buffer and 1 µl DNase I**. Resuspend the magnetic beads by pipetting or vortexing.

    Note: If total nucleic acid (both DNA and RNA) are desired, skip the DNase I digestion step and proceed the wash step starting from step 20.

17. **Incubate at room temperature (22-25°C) for 10 minutes.**

18. **Add 275 µl MRW Buffer to the tube and mix throughly by pipetting up and down for 20 seconds.** Incubate 5 minutes at room temperature.

19. **Place the plate on magnet stand to collect the magnetic beads. Wait until the magnetic beads form a pellet in the side of the wells (if using MSD-01) or until the solution is clear(if using another magnet)** Carefully remove and discard the cleared supernatant.

20. **Remove the plate from magnet and add 300 µl RWB Buffer**. Resuspend the magnetic beads by vortexing.

21. **Place the plate on magnet stand to collect the magnetic beads. Wait until the magnetic beads form a pellet in the side of the wells (if using MSD-01) or until the solution is clear(if using another magnet)** Carefully remove and discard the cleared supernatant

22. **Leave the plate on the magnet to air dry the magnetic beads for 5-10 minutes**. Remove any residue liquid from tube by pipetting.

23. Remove the plate from magnetic separation device. Add 50-100 ul DEPC-treated water and resuspend the beads by pipetting.

24. Incubate 5-10 minutes at room temperature. Place the plate onto magnet to magnetize the Mag-Bind® particles. **Wait until the magnetic beads form a pellet in the side of the wells (if using MSD-01) or until the solution is clear(if using another magnet)**

25. Transfer the cleared supernatant containing purified RNA to a new 96-well microplate.

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.
	MRPL and RWB Wash Buffer were not prepared correctly.	Prepare the MRPL and RWB Wash Buffer by adding isopropanol or absolute ethanol according to instructions.
	Loss of magnetic particles during procedure	Be careful not remove the magnetic particles during the procedure.
No RNA eluted	RWB Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare RWB Wash Buffer Concentrate as instructed.
Problem with downstream application	Insufficient RNA was used	1. RNA in the sample already degraded. Do not freeze and thaw the sample more than once. And do not store at room temperature too long. 2. Quantify the purified RNA accurately and use sufficient RNA.
Carryover of the magnetic beads in the elution	Carryover the magnetic beads in the eluted RNA will not effect downstream applications.	To remove the carryover magnetic particles from the eluted RNA, simply magnetize the magnetic particles and carefully transfer the RNA eluate to a new microfuge tube.