# Mag-Bind® Plant RNA Kit
# Mag-Bind® Plant RNA 96 Kit

## Table of Contents




**Manual Revision: July 2013**


OMEGA bio-tek

Innovations in nucleic acid isolation

# Introduction

The Mag-Bind® Plant RNA Kit and the Mag-Bind® Plant RNA 96 Kit allow rapid and reliable isolation of high-quality total cellular RNA from a wide variety of plant species and tissues. Total RNA from 50 mg of sample can be processed in less than 1 hour. The system uses an innovative Mag-Bind® plant RNA technology to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from plant lysate. The Mag-Bind® Plant RNA Kit provides high-quality RNA. Purified RNA is suitable for all major downstream applications such as RT-PCR, and microarray analysis. Since this kit uses magnetic particles, the protocol can be easily adapted to most robotic liquid handling instruments.

**New in this Edition:**

- Proteinase K is now supplied in a liquid form eliminating the resuspension step to prior to use.
- Proteinase K Solution can also be stored at room temperature for 12 months.
- Proteinase Storage Buffer is no longer included in the kit.

# Kit Contents

| Product Number | M6828-00 | M6828-01 |
|---|---|---|
| Purifications | 5 preps | 50 preps |
| Mag-Bind® Particles CNR | 55 µL | 550 µL |
| RXP Buffer | 5 mL | 35 mL |
| LPX Binding Buffer | 2 mL | 15 mL |
| RNA Wash Buffer II | 5 mL | 25 mL |
| VHB Buffer | 4.4 mL | 26.4 mL |
| DNase I Digestion Buffer | 1.5 mL | 15 mL |
| DNase I | 12 µL | 110 µL |
| RNA Elution Buffer | 600 µL | 10 mL |
| Proteinase K Solution (10 mg/mL) | 110 µL | 1.1 mL |
| User Manual | ✓ | ✓ |

| Product Number | M6927-00 | M6927-01 |
|---|---|---|
| Purifications | 1 x 96 | 4 x 96 |
| Mag-Bind® Particles CNR | 1.1 mL | 4.4 mL |
| RXP Buffer | 70 mL | 250 mL |
| LPX Binding Buffer | 25 mL | 100 mL |
| RNA Wash Buffer II | 50 mL | 200 mL |
| VHB Buffer | 26.4 mL | 110 mL |
| DNase I Digestion Buffer | 25 mL | 100 mL |
| DNase I | 210 µL | 840 µL |
| RNA Elution Buffer | 15 mL | 60 mL |
| Proteinase K Solution (10 mg/mL) | 2.2 mL | 9 mL |
| User Manual | ✓ | ✓ |

## Storage and Stability

Most components of the Mag-Bind® Plant RNA Kit and the Mag-Bind® Plant RNA 96 Kit are stable for at least 12 months from date of purchase when stored at 22-25°C. Mag-Bind® Particles CNR should be stored at 2-8°C. Proteinase K and DNase I should be store at -20°C for long-term storage.

# Preparing Reagents

- Dilute VHB Buffer with 100% ethanol as follows and store at room temperature.

| Kit | 100% Ethanol to be Added |
|---|---|
| M6828-00 | 5.6 mL |
| M6828-01 | 33.6 mL |
| M6927-00 | 33.6 mL |
| M6927-01 | 140 mL |

- Dilute RNA Wash Buffer II with 100% ethanol as follows and store at room temperature.

| Kit | 100% Ethanol to be Added |
|---|---|
| M6828-00 | 20 mL |
| M6828-01 | 100 mL |
| M6927-00 | 200 mL |
| M6927-01 | 800 mL |

- Dilute LPX Binding Buffer with 100% ethanol as follows and store at room temperature.

| Kit | 100% Ethanol to be Added |
|---|---|
| M6828-00 | 2 mL |
| M6828-01 | 15 mL |
| M6927-00 | 25 mL |
| M6927-01 | 100 mL |

## Mag-Bind® Plant RNA Protocol - 1.5 mL Tube Format (M6828)

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 gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents.

**Materials and Equipment to be Provided by User:**

- Centrifuge capable of 13,000 x *g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- Equipment for disrupting plant tissue (MM300 Mixer Mill or Geno/Grinder 2000)
- Mortar and pestle (for manual tissue disruption)
- Magnetic Separation Device for 1.5 mL microcentrifuge tubes (Cat# MSD-02)
- Incubator or water bath capable of 55°C
- Vortex

**Before Starting**

- Prepare Buffers according to the Preparing Reagents Section on Page 4
- Set an incubator to 55°C

**Manual sample disruption:**
To prepare samples, collect a fresh plant sample in a 30 mL mortar and freeze in liquid nitrogen. Grind the tissue using a clean pestle. Allow the liquid nitrogen to evaporate and transfer the sample into a 1.5 mL microcentrifuge tube. Immediately proceed with the RNA isolation protocol on Page 6.

**Mechanical tissue disruption:**
Place the plant sample into a stainless steel grinding jar with the appropriate steel beads. Freeze the sample in the stainless steel grinding jar for 1 minute with liquid nitrogen. Immediately attach the grinding jar onto the clamps of the tissue grinder. Grind the sample at 30Hz for 1-2 minutes. Immediately proceed with the RNA isolation protocol on Page 6.

1. Collect frozen ground plant sample (start with 30-50 mg) in a 1.5 mL microcentrifuge tube.

   **Note:** Keep the sample frozen until adding RXP Buffer.

2. Add 600 µL RXP Buffer. Vortex at maximum speed for 30 seconds.

3. Incubate at 55°C for 3 minutes.

4. Centrifuge at maximum speed for 10 minutes.

   **Note:** Compact pellets will form in the tubes but some particles may float.

5. Carefully transfer 400 µL cleared supernatant to a new 1.5 mL microcentrifuge tube.

   **Note:** Do not disturb the pellet or transfer any debris as it may contain proteins and DNA.

6. Add 400 µL LPX Binding Buffer. Vortex at maximum speed for 30 seconds.

   **Note:** LPX Binding Buffer must be diluted with 100% ethanol before use. Please see Page 4 for instructions.

7. Add 10 µL Mag-Bind® Particles CNR and 20 µL Proteinase K Solution. Vortex at maximum speed for 30 seconds.

8. Let sit at room temperature for 5 minutes. Mix the sample a few times during the incubation by gently inverting the tube.

9. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit for 5 minutes or until the lysate is clear.

10. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

11. Remove the tube from the magnetic separation device.

12. Add 500 µL VHB Buffer. Vortex at maximum speed to completely resuspend the Mag-Bind® Particles CNR.

    **Note:** VHB Buffer must be diluted with 100% ethanol before use. Please see Page 4 for instructions.

13. Let sit at room temperature for 2 minutes.

14. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit for 5 minutes or until the sample is clear.

15. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

16. Remove the tube from the magnetic separation device.

17. Add 700 µL RNA Wash Buffer II. Vortex at maximum speed to completely resuspend the Mag-Bind® Particles CNR.

    **Note:** RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 4 for instructions.

18. Let sit at room temperature for 2 minutes.

19. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit for 5 minutes or until the sample is clear.

20. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

21. Leave the tube on the magnetic separation device for 5-10 minutes to air dry the Mag-Bind® Particles CNR. Remove any residual liquid with a pipettor.

22. Remove the tube from the magnetic separation device.

23. Add 200 µL DNase mixture (198 µL DNase I Digestion Buffer and 2 µL DNase I). Mix thoroughly by pipetting up and down 10 times or inverting the tube 10 times.

24. Let sit at room temperature for 15 minutes to digest genomic DNA.

25. Add 500 µL RNA Wash Buffer II. Vortex at maximum speed to completely resuspend the Mag-Bind® Particles CNR.

26. Let sit at room temperature for 5 minutes.

27. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit for 5 minutes or until the sample is clear.

28. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

29. Remove the tube from the magnetic separation device.

30. Add 700 µL RNA Wash Buffer II. Vortex at maximum speed to completely resuspend the Mag-Bind® Particles CNR.

31. Let sit at room temperature for 2 minutes.

32. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit for 5 minutes or until the sample is clear.

33. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

34. Leave the tube on the magnetic separation device for 5-10 minutes to air dry the Mag-Bind® Particles CNR. Remove any residual liquid with a pipettor.

35. Remove the tube from the magnetic separation device.

36. Add 100 µL RNA Elution Buffer. Vortex at maximum speed to completely resuspend the Mag-Bind® Particles CNR.

37. Let sit at room temperature for 5 minutes.

38. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit for 5 minutes or until the sample is clear.

39. Transfer the cleared supernatant containing purified RNA to a new 1.5 mL microcentrifuge tube and store the RNA at -70°C.

## Mag-Bind® Plant RNA Protocol - 96-well Plate Format (M6927)

**Materials and Equipment to be Provided by User:**

- Centrifuge capable of 4,000 x *g* with swinging-bucket rotor for 96-well plates
- Sealing film
- 2.2 mL KF Deep-well Plate (Recommended: Cat# OME1799)
- 100% ethanol
- 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-01B)
- Incubator capable of 55°C

**Before Starting**

- Prepare Buffers according to the Preparing Reagents Section on Page 4
- Set an incubator to 55°C

**Manual sample disruption:**
To prepare samples, collect a fresh plant sample in a 30 mL mortar and freeze in liquid nitrogen. Grind the tissue using a clean pestle. Allow the liquid nitrogen to evaporate and transfer the sample into a 96-well deep-well plate. Immediately proceed with the RNA isolation protocol below.

**Mechanical tissue disruption:**
Place the plant sample into a stainless steel grinding plate with the appropriate steel beads. Freeze the sample in the stainless steel grinding plate for 1 minute with liquid nitrogen. Immediately attach the grinding plate onto the clamps of the tissue grinder. Grind the sample at 30Hz for 1-2 minutes. Immediately proceed with the RNA isolation protocol below.

1. Collect frozen ground plant sample (start with 30-50 mg) in a deep-well plate.

   **Note:** Keep the sample frozen until adding RXP Buffer.

2. Add 600 µL RXP Buffer. Seal the plate. Vortex at maximum speed for 1 minute.

3. Incubate at 55°C for 3 minutes.

4. Centrifuge at maximum speed for 10 minutes to pellet cell debris.

   **Note:** Compact pellets will form in the wells but some particles may float.

5. Carefully transfer 400 µL cleared supernatant to a new 2.2 mL KF Deep-well Plate.

   **Note:** Do not disturb the pellet or transfer any debris as it may contain proteins and DNA.

6. Add 400 µL LPX Binding Buffer. Seal the plate. Vortex at maximum speed for 30 seconds.

   **Note:** LPX Binding Buffer must be diluted with 100% ethanol before use. Please see Page 4 for instructions.

7. Add 10 µL Mag-Bind® Particles CNR and 20 µL Proteinase K Solution. Seal the plate. Vortex at maximum speed for 30 seconds.

8. Let sit at room temperature for 5 minutes. Mix the samples a few times during the incubation by gently shaking the plate.

9. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit for 5 minutes or until the lysate is clear.

10. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

11. Remove the plate from the magnetic separation device.

12. Add 500 µL VHB Buffer. Seal the plate. Vortex at maximum speed to completely resuspend the Mag-Bind® Particles CNR.

    **Note:** VHB Buffer must be diluted with 100% ethanol before use. Please see Page 4 for instructions.

13. Let sit at room temperature for 2 minutes.

14. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit for 5 minutes or until the sample is clear.

15. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

16. Remove the plate from the magnetic separation device.

17. Add 700 µL RNA Wash Buffer II. Seal the plate. Vortex at maximum speed to completely resuspend the Mag-Bind® Particles CNR.

    **Note:** RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 4 for instructions.

18. Let sit at room temperature for 2 minutes.

19. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit for 5 minutes or until the sample is clear.

20. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

21. Leave the plate on the magnetic separation device for 5-10 minutes to air dry the Mag-Bind® Particles CNR. Remove any residual liquid with a pipettor.

22. Remove the plate from the magnetic separation device.

23. Add 200 µL DNase mixture (198 µL DNase I Digestion Buffer and 2 µL DNase I). Mix thoroughly by pipetting up and down 10 times or inverting the plate 10 times.

24. Let sit at room temperature for 15 minutes to digest genomic DNA.

25. Add 500 µL RNA Wash Buffer II. Seal the plate. Vortex at maximum speed to completely resuspend the Mag-Bind® Particles CNR.

26. Let sit at room temperature for 5 minutes.

27. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit for 5 minutes or until the sample is clear.

28. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

29. Remove the plate from the magnetic separation device.

30. Add 700 µL RNA Wash Buffer II. Vortex at maximum speed to completely resuspend the Mag-Bind® Particles CNR.

31. Let sit at room temperature for 2 minutes.

32. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit for 5 minutes or until the sample is clear.

33. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

34. Leave the plate on the magnetic separation device for 5-10 minutes to air dry the Mag-Bind® Particles CNR. Remove any residual liquid with a pipettor.

35. Remove the plate from the magnetic separation device.

36. Add 100 µL RNA Elution Buffer. Seal the plate. Vortex at maximum speed to completely resuspend the Mag-Bind® Particles CNR.

37. Let sit at room temperature for 5 minutes.

38. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit for 5 minutes or until the sample is clear.

39. Transfer the cleared supernatant containing purified RNA to a new 96-well plate and store the RNA at -70°C.

# Troubleshooting Guide

Please use this guide to solve any problems that may arise. If for any reason you need further assistance, please contact our technical support staff at our **Toll Free Number at 1-800-832-8896.**

**Possible Problems and Suggestions**

| Troubleshooting Guide | | |
|---|---|---|
| **Problem** | **Likely Cause** | **Suggestions** |
| **Low RNA yields** | Incomplete re-suspension of magnetic particles | Resuspend the magnetic particles by vortexing before use. |
| | RNA degraded during sample storage | Make sure the sample is properly stored and the samples are processed immediately after collection or removal from storage. |
| | VHB Buffer, LPX Binding Buffer, and RNA Wash Buffer II not prepared correctly | Prepare VHB Buffer, LPX Binding Buffer, and RNA Wash Buffer II as instructed on Page 4. |
| | Loss of magnetic particles during procedure | Be careful not remove the magnetic particles during the procedure. |
| **No RNA eluted** | VHB Buffer and RNA Wash Buffer II are not diluted with 100% ethanol. | Prepare VHB Buffer and RNA Wash Buffer II as instructed on Page 4. |
| **Problem with downstream application** | Insufficient RNA was used | RNA in the sample already degraded. Do not freeze and thaw the sample more than once. Do not store at room temperature too long. |
| **Carryover of the magnetic particles during elution** | Carryover of magnetic particles 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 microcentrifuge tube. |

**Notes:**

**Notes:**