MAGBIO

"WE MAKE NGS BETTER"

HighPrep™ Plant RNA Plus

Catalog Nos. HPPP-R10, HPPP-R96, HPPP-R96X4
Manual Revision v1.0

- Total RNA Isolation from a variety of plants and fungi
- Magnetic-beads based chemistry

PROTOCOL

Contents

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Product Description

The HighPrep™ Plant RNA Plus Kit is specially designed for purifying RNA from a wide range of plant and fungi species. The kit uses a special lysis condition with HighPrep™ magnetic particles technology to isolate high-quality RNA. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including real time PCR, Southern blotting, SNP analysis, NGS, and hybridization applications. The HighPrep™ Plant RNA Plus Kit can be adapted to high-throughput liquid handling work stations.

Advantages

- Fast and easy processing using a magnetic bead system.
- Robust lysis system (chemical lysis combined with a mechanical homogenization).
- Isolate high-quality total RNA from a variety of fungi and plant species.

Process

Plant samples are disrupted in a homogenizer/bead-based milling equipment. RL Buffer is added to lyse the samples. The supernatant is then transferred to a new processing plate where MAG-R5 Particles are added to bind to the DNA. Following a few wash steps, DNA is eluted from the MAG-R5 Particles for downstream application.

Kit Contents and Storage

HighPrep™ Plant RNA Plus Kit Catalog No.	**HPPP-R10**	**HPPP-R96**	**HPPP-R96X4**	**STORAGE**
Number of Preps	10	96	384	
RL Buffer	8 mL	60 mL	240 mL	15-25°C
PRW1 Buffer[1]	4.5 mL	36 mL	150 mL	15-25°C
PRW2 Buffer[1]	5 mL	50 mL	100 mL x 2	15-25°C
RNA Elution Buffer	1.5 mL	10 mL	50 mL	15-25°C
DNase I Digestion Buffer	1.1 mL	10 mL	40 mL	15-25°C
MAG-R5 Particles	0.22 mL	2 mL	8 mL	2-8°C
DNase I	0.025 mL	0.2 mL	0.8 mL	-20°C

[1] Ethanol must be added prior to use. See Preparation of Reagents

Stability

All components are stable for 14 months when stored accordingly.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). MSDS can be downloaded from the "Product Documents" tab when viewing the product kit.

Preparation of Reagents

Prepare the following components for each kit before use:

Catalog No.	Component	Add 100% Ethanol	Storage
HPPP-R10	PRW1 Buffer	3 mL	Room Temp 15-25°C
	PRW2 Buffer	20 mL	Room Temp 15-25°C
Components are stable for 14 months when stored closed at room temperature			

Catalog No.	Component	Add 100% Ethanol	Storage
HPPP-R96	PRW1 Buffer	24 mL	Room Temp 15-25°C
	PRW2 Buffer	200 mL	Room Temp 15-25°C
Components are stable for 14 months when stored closed at room temperature			

Catalog No.	Component	Add 100% Ethanol	Storage
HPPP-R96X4	PRW1 Buffer	100 mL	Room Temp 15-25°C
	PRW2 Buffer	400 mL x 2	Room Temp 15-25°C
Components are stable for 14 months when stored closed at room temperature			

Prepare a fresh aliquot of Lysis Buffer supplemented with DTT according to the following table:

Reagent	Volume
RL Buffer	600 µl
2M DTT	12 µl

Note: Only 600 µl of the Lysis Buffer with DTT is required for each sample disruption.

Amount of starting material

Use the amount of starting material indicated in Table 1.

Table 1. Amount of starting material for HighPrep™ Plant RNA Plus kit

Sample	Amount
Plant Tissue	up to 50 mg

Working in RNase Free Conditions

RNases are present everywhere and some general precautions should be followed in order to avoid the introduction of contaminating nucleases. The most common sources of RNase contamination are hands, dust particles, and contaminated laboratory instruments, solutions, and glassware. The following procedures should be followed to limit RNase contamination when working with RNA:

- Always wear gloves while working and change gloves frequently.
- Refrain from using reagents, consumables and equipment that are in common use for other general lab processes.
- Use dedicated RNase free equipment such as pipettes, pipette tips, gels boxes, etc.
- Work in a separate room, fume hood or lab space if available.
- Use plastic, disposable consumables that are certified RNase free.
- Purchase reagents, such as commonly used buffers and water, that are certified RNase free. Prepare small individual aliquots of such buffers to avoid repeated transfer out of stock buffers. This lowers the risk of contamination of the stock solution.
- Wipe down work surfaces with commercial RNase inhibiting surfactant solutions or 70% ethanol before starting work.

HighPrep™ Plant RNA Plus Kit

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) from each product supplier.

- ☐ Nuclease-free 1.5~2 ml microcentrifuge tubes
- ☐ Magnetic separation device
- ☐ Water bath, incubator, or heating block capable of 65°C
- ☐ Equipment for disrupting plant tissue (Geno/Grinder 2010 or MM300 Mixer Mill etc)
- ☐ 100% Ethanol
- ☐ Vortex
- ☐ 2M DTT

Things to do before starting

- ☐ Prepare PRW1 Buffer and PRW2 Buffer according to the instructions in the Preparation of Reagents Section.
- ☐ Preset water bath, incubator, or heating block to 65°C.
- ☐ Preheat Elution Buffer to 65°C.
- ☐ Suspend MAG-R5 Particles by vortexing.
- ☐ Prepare a fresh aliquot of RL Buffer supplemented with DTT according to the instructions in the Preparation of Reagents Section

Protocol

1. Disrupt the tissue.

A) Disrupt the sample manually:

Recommend using 10–50 mg of fresh plant samples or 10–20 mg of seed samples.

1. **Add liquid nitrogen to a clean mortar.**
2. **Freeze the plant tissue by placing it in the liquid nitrogen in the mortar.**
3. **Grind the tissue thoroughly using a clean pestle, then allow the liquid nitrogen to evaporate.**
4. **Add 600 µL of RL Buffer with DTT to a 1.5-mL microcentrifuge tube.**
5. **Transfer up to 50 mg of homogenized sample into the tube containing RL Buffer with DTT, then mix thoroughly by vigorously vortexing for 10–20 seconds.**
   ⚠ *Transfer the ground tissue to the Lysis Buffer with DTT as quickly as possible to avoid RNA degradation.*
6. **Incubate at 65°C for 5 minutes.**
7. **Centrifuge for 10 minutes at maximum speed (≥ 16,000 × g) to clear the plant lysate.**
8. **Transfer 400 µl of the supernatant to a clean 1.5-mL microcentrifuge tube (manual purification) or to the well of a 96-deep well plate (automated purification).**

B) Disrupt the sample mechanically:

Plant tissue can be homogenized with a rotor-stator, bead mill, or high-throughput homogenizers. We recommend bead mill or high-throughput homogenizers paired with stainless steel beads. When using beads for homogenization, ensure that the correct tubes are used, which typically have thicker walls and a screw cap with an O-ring. High-throughput homogenizers offer an appropriate method for handling 96 samples simultaneously. For low throughput homogenization it is possible to use 1.5 – or 2.0–ml microcentrifuge tubes.

For mechanical disruption with bead mill or high-throughput homogenizers, we recommend:
- 10–50 mg of plant sample in single microtubes
- 10–20 mg of plant sample in racked 96-well collection microtubes
- 10–20 mg of plant seeds as starting material in racked 96-well collection or single microtube

For mechanical disruption with a rotor-stator homogenizer, we recommend:
- Up to 50 mg of plant sample in a single microcentrifuge tube
- Up to 20 mg of plant seeds as starting material in a single microcentrifuge tube

1. **(Optional) Add a stainless-steel bead to a tube if required for your method.**
2. **Add 600 µL of RL Buffer with DTT to the tube.**
3. **Place the plant tissue in the tube.**
4. **Homogenize the sample according to the manufacturer recommendations.**
5. **After sample homogenization, incubate the sample at 56°C for 5 minutes.**
6. **Centrifuge for 10 minutes at maximum speed (≥ 16,000 × g for tubes or 3000–4000 × g for plates) to clear the plant lysate.**
7. **Transfer 400 µl of the supernatant to a clean 1.5-mL microcentrifuge tube (manual purification) or to the well of a 96-deep well plate (automated purification)**

2. **Add 20 µl of MAG-R5 Particles to the supernatant collected above and vortex at maximum speed for 10 seconds, or pipette 20 times.**

   ⚠ *Complete resuspension of the MAG-R5 Particles is crucial for obtaining high purity RNA.*

3. **Add 400 µl of 100% Ethanol to the supernatant collected above and vortex at maximum speed for 10 seconds, or pipette 20 times. Incubate the sample tube at room temperature for 5 minutes.**

4. **Place the sample on the magnetic separation device for 2 minutes or until the solution completely clears and the beads are collected against the magnet. Remove and discard the cleared supernatant. Do not disturb the magnetic beads.**

5. **Add 600 µl of PRW1 Buffer and resuspend the magnetic beads by vortexing at maximum speed for 10 seconds, or pipette mix 20 times.**

   ⚠ *PRW1 Buffer must be diluted with ethanol before use.*

6. **Place the sample back on the magnetic separation device for 2 minutes or until the solution completely clears and the beads are collected against the magnet. Remove and discard the cleared supernatant. Do not disturb the magnetic beads.**

7. **Add 600 µl of PRW2 Buffer and resuspend the magnetic beads by vortexing at maximum speed for 10 seconds, or pipette mix 20 times.**

⚠ *PRW1 Buffer must be diluted with ethanol before use.*

6. **Place the sample back on the magnetic separation device for 2 minutes or until the solution completely clears and the beads are collected against the magnet. Remove and discard the cleared supernatant. Do not disturb the magnetic beads.**

7. **Add 600 µl of PRW2 Buffer and resuspend the magnetic beads by vortexing at maximum speed for 10 seconds, or pipette mix 20 times.**

   ⚠ *PRW2 Buffer must be diluted with ethanol before use.*

8. **Place the sample on the magnetic separation device for 2 minutes or until the solution completely clears and the beads are collected against the magnet. Remove and discard the cleared supernatant. Do not disturb the magnetic beads.**

9. **Leave the sample on the magnetic separation device for 5 minutes to air dry the magnetic beads.**

10. **While the samples are drying, prepare the DNase I mixture. For each sample, gently mix 98 µl of DNase I Digestion Buffer and 2 µl of DNase I.**

11. **Remove the sample from the magnetic separation device and add 100 µl of the DNase I mixture to each of the samples. Mix by pipetting up and down to fully resuspend the magnetic beads. Incubate the sample at 37°C for 15 minutes.**

    ⚠ *Avoid extensive vortex or pipetting as this may denature the DNase I.*

12. **To rebind the RNA, add 600 µl of PRW2 Buffer to the sample and resuspend the magnetic beads by vortexing at maximum speed for 10 seconds, or pipette mix 20 times. Incubate the sample tube at room temperature for 2 minutes.**

    ⚠ *PRW2 Buffer must be diluted with ethanol before use. Complete resuspension of the magnetic beads is critical for obtaining high quality RNA.*

13. **Place the sample on the magnetic separation device for 2 minutes or until the solution completely clears and the beads are collected against the magnet. Remove and discard thecleared supernatant. Do not disturb the magnetic beads.**

14. **Add 600 µl of PRW2 Buffer to the sample and resuspend the magnetic beads by vortexing at maximum speed for 10 seconds, or pipette mix 20 times.**

    ⚠ *PRW2 Buffer must be diluted with ethanol before use.*

15. **Place the sample on the magnetic separation device for 2 minutes or until the solution completely clears and the beads are collected against the magnet. Remove and discard the cleared supernatant. Do not disturb the magnetic beads.**

16. **(Optional) Repeat Steps 14-15 for one more bead wash.**

17. **Keep sample on the magnetic separation device and air dry the magnetic beads by incubating at room temperature for 5 minutes. Remove any residual liquid with a fine pipette tip.**

⚠ *It is critical to completely remove all liquid from each sample tube.*

18. Add 50 - 100 µl of RNA Elution Buffer and completely resuspend the magnetic beads by vortexing at maximum speed for 10 seconds, or pipette mix 20 times. Incubate for 5 minutes at room temperature.

⚠ *Incubating the sample at 65°C may increase the yield.*

19. Place the sample back on the magnetic separation device and wait 2-5 minutes or until the magnetic beads are completely cleared from the Elution Buffer.

20. Transfer the eluate (cleared supernatant) containing purified RNA to a new 1.5ml nuclease free tube, and store purified RNA at -80 °C.

Troubleshooting guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via:
Phone: 1-855-262-4246 (in US), outside US, 1-919-719-0665
Email: support@magbiogenomics.com

Symptoms	Possible Causes	Comments
Low RNA yield	The sample was not stored properly, causing RNA degradation	Use fresher samples. Ensure that samples are processed immediately after collection or removal from storage. If immediate processing is not possible, flash freeze the samples in liquid nitrogen, then store at –80°C.
	The sample was not sufficiently homogenized.	To adequately disrupt the cell wall and therefore release the RNA, it is important to homogenize the sample thoroughly. Efficient homogenization and lysis of the plant cells increases the RNA yield.
	Ethanol was not added to the lysate.	Ensure that 400 µL of ethanol was added to the lysate to bind the RNA to the MAG-R5 Particles.
	The RNA rebinding step was not performed.	Ensure that PRW2 Buffer is added after the DNase I treatment.
	Ensure that samples are processed immediately	freeze the samples in liquid nitrogen, then store
	Beads were lost during purification.	Avoid disturbing the MAG-R5 Particles during aspiration of supernatant
	Ethanol is not added into PRW1 and PRW2 Buffer	Add absolute 100% Ethanol to PRW1 and PRW2 Buffer.
MAG- R5 Particles do not completely clear from solution	Too short of magnetizing time.	Increase collection time on the magnet.
Problems in downstream applications	Ethanol carry-over	Dry the MAG-R5 Particles completely before elution.

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use. Ensure that a suitable lab coat, disposable gloves, and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs).

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