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MasterPure™ Plant RNA Purification Kit

Cat. Nos. MPR09010 and MPR09100

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1. Introduction

The MasterPure™ Plant RNA Purification Kit provides all of the reagents necessary to isolate highly purified RNA from a wide variety of plant species. This kit uses an optimized Plant Tissue and Cell Lysis Solution to inactivate and remove polyphenols and polysaccharides, while releasing nucleic acids and inactivating ribonucleases. Lysis is followed by a quick precipitation Part A and a rapid desalting process to remove contaminating macromolecules without the use of toxic organic solvents.[1] These contaminants in plant nucleic acid preparations can interfere with downstream applications. The MasterPure-purified RNA can be used in many applications including amplification, hybridization, RNase protection, and RT-PCR.

2. Kit Contents

MasterPure™ Plant RNA Purification Kit Contents

The MasterPure RNA Purification Kit is available in two sizes (10 purifications and 100 purifications).

Cat. #	Concentration	Quantity
MPR09010		10 Purifications
Plant Tissue and Cell Lysis Solution		6 ml
2X T and C Lysis Solution		2 ml
MPC Protein Precipitation Reagent		4.5 ml
RNase-Free Water		2.2 ml
RNase-Free DNase I	@ 1 U/µl	50 µl
Proteinase K	@ 50 µg/µl	10 µl
RiboGuard™ RNase Inhibitor	@ 40 U/µl	30 µl
100 mM DTT		60 µl
10X DNase Buffer		200 µl
MPR09100		100 Purifications
Plant Tissue and Cell Lysis Solution		60 ml
2X T and C Lysis Solution		20 ml
MPC Protein Precipitation Reagent		45 ml
RNase-Free Water		22 ml
RNase-Free DNase I	@ 1 U/µl	500 µl
Proteinase K	@ 50 µg/µl	100 µl
RiboGuard™ RNase Inhibitor	@ 40 U/µl	300 µl
100 mM DTT		600 µl
10X DNase Buffer		2 ml

3. Product Specifications

Storage: Store the RNase-Free DNase I, Proteinase K, RiboGuard™ RNase Inhibitor, dithiothreitol (DTT), and 10X DNase Buffer at –20°C in a freezer without a defrost cycle. Store the remainder of the kit at room temperature.

Storage Buffers: RNase-Free DNase I is supplied in a 50% glycerol solution containing 10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, and 10 mM $CaCl_2$; Proteinase K is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 10 mM $CaCl_2$, 0.1% Triton® X-100, and 1 mM DTT; RiboGuard RNase Inhibitor is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 10 mM DTT, and 0.1 mM EDTA.

Quality Control: The MasterPure Plant RNA Purification Kit is function-tested by purifying RNA from alfalfa sprouts. RNA quality and yield are assayed by agarose gel electrophoresis, spectrophotometry, and as a template for RT-PCR.

4. Related Products

The following products are also available:

- MasterPure™ Complete DNA and RNA Purification Kits
- MasterPure™ Plant Leaf DNA Purification Kit
- QuickExtract™ RNA Extraction Kits
- MasterAmp™ High Fidelity RT-PCR Kits
- MasterAmp™ RT-PCR Kits for High Sensitivity
- MMLV Reverse Transcriptase 1st-Strand cDNA Synthesis Kit
- MonsterScript™ 1st-Strand cDNA Synthesis Kit
- Message*BOOSTER*™ cDNA Synthesis Kit for qPCR
- MasterAmp™ PCR Optimization Kits
- FailSafe™ PCR System

5. General Considerations

1. **Tissue Sources:** We have used the MasterPure Plant RNA Purification Kit to isolate RNA from a variety of plant tissue sources including: grape, strawberry, raspberry, banana, tomato, corn, and soybean leaves; alfalfa sprouts, soybean and corn seedlings and mature roots, as well as pine needles. Tissues other than those mentioned here are likely to be compatible with the kit.
2. **Age of Tissue:** Young, fresh tissues are ideal for isolating intact RNA. The content of polyphenols increases with the age of the tissue, making RNA preparations more difficult with older samples.
3. **Tissue Homogenization:** Fresh or frozen tissue can be used. Harvest the plant tissue and submerge in liquid nitrogen as quickly as possible to prevent RNA degradation. Store at –70°C until use. The simplest method to prepare samples for lysis is to grind the tissue to a fine powder in liquid nitrogen with a cold mortar and pestle (chilled to –20°C or kept on dry ice). Do not allow the sample to thaw during processing. Other types of motorized tissue homogenizers can also be used effectively. The amount of time and force needed to disrupt different plant tissue samples down to single-cell

homogenates will vary widely.

4. **Sample Size:** You can purify nucleic acid from samples of various sizes by proportionally adjusting the amount of reagents to the amount of starting material. With larger samples, centrifugation conditions (time and speed) may also need to be adjusted.
5. **Proteinase K and Heat Treatment:** We recommend including the Proteinase K treatment (Part A, Steps 2-4) to increase the efficiency of lysis and improve yield, though for some samples this treatment is unnecessary. If you wish to minimize the time of purification, you may determine if Proteinase K treatment is required. Certain tissues may swell or be adversely affected by prolonged incubation at 56°C; for these tissues, we recommend shortening the incubation to 5 minutes or eliminating it.
6. **Nuclease Treatment:** We highly recommend treating the RNA Preparation with DNase I as per protocol. The final clean-up steps following the treatment significantly benefit the purity of the resulting RNA.
7. **Reducing Agents:** DTT is added to the lysis buffer in Part A, Step 2. However, a slightly higher yield may be obtained from some tissues if 3 µl of β-mercaptoethanol is substituted for DTT.
8. **Working with RNA:** RNases are stable and ubiquitous. Care must be taken to avoid the introduction of exogenous RNases during purification. Always wear gloves when handling the kit reagents and the sample tissues.

6. RNA Purification Protocols

A. Lysis of Tissue Samples

Thoroughly mix all solutions to ensure uniform composition before use.

1. Collect 25-100 mg of fresh weight or frozen plant tissue and homogenize by quick-freezing in liquid nitrogen and grinding to a fine powder with a prechilled mortar and pestle. Other tissue homogenization methods can also be used. See General Considerations.
2. Allow the liquid nitrogen to dissipate, but do not allow the sample to thaw.

   To each sample, quickly add:

600	µl	Plant Tissue and Cell Lysis Solution
6	µl	100 mM DTT
1	µl	Proteinase K

3. Mix by vortexing vigorously for 1 minute.
4. Incubate at 56°C for 15 minutes. Mix by vortexing every 5 minutes for 15-30 seconds to improve the yield of nucleic acids.

   Note: *For some tissues, shortening this step may be optimal. See General Considerations.*
5. Pellet the debris by centrifugation for 5 minutes at ≥10,000 x g at room temperature and transfer the clarified supernatant to a new tube. Try to minimize the carryover of particulates.
6. Place the samples on ice for 3-5 minutes.

B. Precipitation of Nucleic Acids

1. Add 250 µl of MPC Protein Precipitation Reagent to the sample and mix by vortexing vigorously for 10 seconds.
2. Pellet the debris by centrifugation at 4°C for 10 minutes at ≥10,000 x g in a microcentrifuge.
3. Transfer the supernatant to a clean microcentrifuge tube and discard the pellet.
4. Add 500 µl of isopropanol to the recovered supernatant. Invert the tube 30-40 times.
5. Pellet the nucleic acids by centrifugation at 4°C for 10 min. at ≥10,000 x g in a microcentrifuge.
6. Carefully pour off the isopropanol without dislodging the total nucleic acid pellet.
7. Remove all of the residual isopropanol.

Note: *The pellet may not be white at this step.*

C. Removal of Contaminating DNA from RNA Preparations

1. Prepare 200 µl of DNase I solution for each sample as follows:

173	µl	RNase-Free Water
20	µl	10X DNase Buffer
5	µl	RNase-Free DNase I
2	µl	RiboGuard RNase Inhibitor

2. Completely resuspend the nucleic acid pellet in 200 µl of DNase I solution.
3. Incubate at 37°C for 10 minutes. Note: Additional incubation (up to 30 minutes) may be necessary to remove all contaminating DNA.
4. Add 200 µl of 2X T and C Lysis Solution; mix by vortexing for 5 seconds.
5. Add 200 µl of MPC Protein Precipitation Reagent; mix by vortexing 10 seconds; place on ice for 3-5 minutes.
6. Pellet the debris by centrifugation at 4°C for 10 minutes at ≥10,000 x g in a microcentrifuge.
7. Transfer the supernatant containing the RNA into a clean microcentrifuge tube and discard the pellet.
8. Add 500 µl of isopropanol to the supernatant. Invert the tube 30-40 times.
9. Pellet the purified RNA by centrifugation at 4°C for 10 minutes in a microcentrifuge.
10. Carefully pour off the isopropanol without dislodging the RNA pellet.
11. Wash twice with 70% ethanol. Centrifuge briefly. Remove all of the residual ethanol with a pipet.
12. Resuspend the RNA in 10-40 µl of RNase-Free Water.
13. Add 1 µl of RiboGuard RNase Inhibitor (optional). Store at –70°C.

7. Troubleshooting RNA Purification

Little or no RNA obtained

1) Create an RNase-free work environment. Change gloves frequently. Do not touch kit components with ungloved hands. Keep tubes closed whenever possible during the purification procedure.
2) Improve tissue homogenization. Inadequate tissue disruption will substantially decrease yields. Use another more vigorous tissue homogenization technique appropriate to your starting material. If fresh tissue was used, quick-freeze it in liquid nitrogen before grinding to a powder. Keep the homogenizer submerged to minimize foaming and rotate the tube. Additional lysis solution can be added to sample.
3) Re-extract the tissue debris with additional lysis buffer. Use more Plant Tissue and Cell Lysis Solution to extract RNA from the tissue debris. To improve yields, add 300 µl of additional Plant Tissue and Cell Lysis Solution to the debris pellet in Part A, Step 5, mix by vortexing for 1 minute and repeat the centrifugation in Part A, Step 5. Pool this new supernatant with the original supernatant from Part A, Step 5 and proceed with the protocol.
4) Increase the efficiency of cell lysis. Mix the homogenate by vigorous vortexing for a full minute, as per protocol. Mix the sample frequently during the Proteinase K treatment to facilitate cell lysis.
5) Decrease the amount of water. Use less water to resuspend precipitated RNA. If the tissue is extremely wet, the tissue samples should be blotted dry before use. Some samples can even be lyophilized before disruption to increase the yield from wet tissues.
6) Ensure that RNA pellet is not lost following isopropanol precipitation. Make certain that the RNA pellet adheres to the microcentrifuge tube during washing of the pellet with 70% ethanol.
7) Recalcitrant tissues. Some problematic tissues with high levels of RNases, phenolics, polysaccharides, or other contaminants require decreased starting amounts of material for adequate purification results. Decreasing the starting material from 25-50 mg (from 100 mg) may improve RNA yield and integrity. Better results may also be obtained by shortening or eliminating the 56°C lysis incubation for some tissues.

A_{260}/A_{280} or A_{260}/A_{230} ratio is too low

1) Decrease the amount of starting material. Use less starting material. Alternatively, dilute the RNA to 300 µl with Tissue and Cell Lysis Solution, and repeat the purification protocol.
2) Perform protein precipitation at 4°C. Cool the sample thoroughly on ice before adding the MPC Protein Precipitation Reagent. The centrifugation steps should be performed at 4°C. The MPC Protein Precipitation Reagent can also be stored at –20°C and used chilled.
3) DNase I Treatment and Final Purification. Be sure to perform the DNase I treatment and cleanup steps (see Part C). Omitting these steps will result in unacceptable absorbance ratios.

Loose protein pellet

1) Chill the sample before protein precipitation. Chill the sample thoroughly on ice before adding the MPC Protein Precipitation Reagent. The centrifugation steps should be performed at 4°C. The MPC Protein Precipitation Reagent can also be stored at –20°C and used chilled. If the pellet remains loose, add 75 µl of additional Protein Precipitation Reagent and centrifuge again. Carefully remove the supernatant to minimize the transfer of precipitated protein. Note that a small degree of transfer is generally not detrimental.

8. Reference

1. Miller, S.A. *et al.,* (1988) *Nucl. Acids Res.* **16**, 1215.

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