microRNA Purification from Plant Tissues Using the ReliaPrep™ miRNA Cell and Tissue Miniprep System

Purify high-quality, amplifiable total RNA, including microRNA, from plant tissue.

Promega Corporation

Sample Type: Corn, soybean and *Arabidopsis* leaf tissue.

Material Required: Fisherbrand® Motorized Tissue Grinder (Fisher Scientific Cat.# 12-1413-61).

Analyses: UV absorbance and QuantiFluor® quantitation, RT-qPCR

Protocol: ReliaPrep™ miRNA Cell and Tissue Miniprep System Technical Manual #TM469.

Further information can be found in Technical Manual #TM469, available at: **www.promega.com/protocols**, or e-mail: **techserv@promega.com**

Protocol

1. Grind plant tissue using liquid nitrogen and mortar and pestle.
2. Add 200µl of LBA + 1-thioglycerol to plant tissue in a 1.5ml microcentrifuge tube.

   Note: Additional LBA + 1-thioglycerol may be added to maximize recoverable lysate volume.
3. Homogenize the tissue using a Fisherbrand® Motorized Tissue Grinder with disposable pestles.
4. Centrifuge the sample at maximum speed for 1 minute to pellet debris. Transfer the cleared homogenate (maximum 200µl) to a clean 1.5ml microcentrifuge tube.
5. Add 130µl of RDB, and vortex for 10 seconds.
6. Centrifuge at 12,000 × *g* for 2 minutes.
7. Transfer homogenate to a new 1.5ml tube.
8. Add 400µl of 100% isopropanol to each cleared homogenate. Mix by vortexing.
9. Transfer homogenate to a ReliaPrep™ Minicolumn. Centrifuge at 12,000 × *g* for 30 seconds.
10. Discard the liquid in the collection tube.
11. Add 500µl of RWA to each column. Centrifuge at 12,000 × *g* for 30 seconds. Discard liquid in the collection tube.
12. Add 500µl of RWA to each column. Centrifuge at 12,000 × *g* for 2 minutes.
13. Transfer column to a 1.5ml Elution Tube.
14. Add 40µl of Nuclease-Free Water to each column. Centrifuge at 12,000 × *g* for 1 minute.
15. Transfer 5µl of DNase I and 5µl of DNase 10X Buffer to each eluate.
16. Incubate 5 minutes at room temperature.
17. Add 150µl of LBA to the samples.

18. Add 300µl of 95% ethanol to the mixture, and vortex for 10 seconds. Transfer mixture to a new ReliaPrep™ Minicolumn.
19. Centrifuge at 12,000 × *g* for 30 seconds. Discard the liquid in the collection tube.
20. Add 500µl of RWA. Centrifuge at 12,000 × *g* for 30 seconds. Discard liquid in the collection tube.
21. Add 500µl of RWA. Centrifuge at 12,000 × *g* for 2 minutes. Discard liquid in the collection tube.
22. Transfer column to a 1.5ml Elution Tube.
23. Add 50µl of Nuclease-Free Water. Centrifuge at 12,000 × *g* for 1 minute.

Results

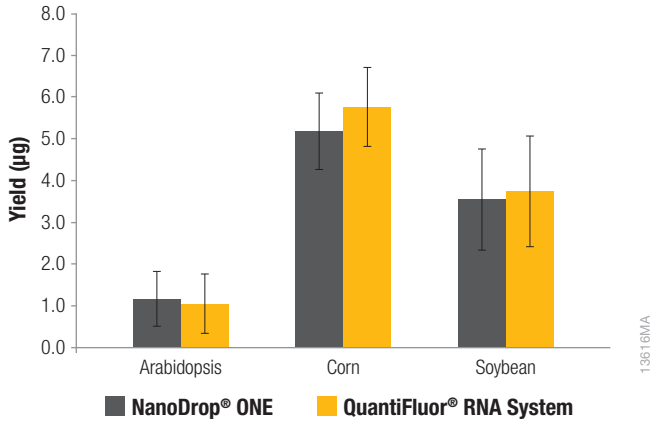

Figure 1. Yields of RNA from plant tissues determined using the NanoDrop® ONE and the QuantiFluor® RNA System (Cat.# E3310) on the Quantus™ Fluorometer (Cat.# E6150). N=3.

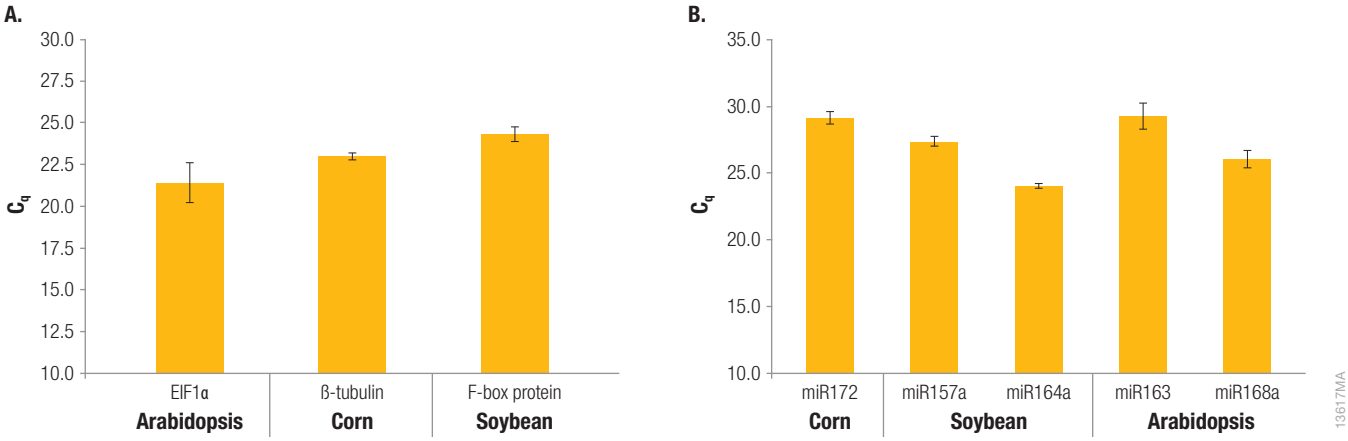

Figure 2. RT-qPCR of purified RNA. Panel A. RT-qPCR of mRNA using plant-specific primers for housekeeping genes with the GoTaq® 1-Step RT-qPCR System (Cat.# A6020). N=3. **Panel B.** RT-qPCR of miRNA using TaqMan® miRNA-specific primers and the TaqMan® microRNA reverse transcription kit followed by qPCR using the GoTaq® Probe qPCR Master Mix (Cat.# A6101). N=3.

Ordering Information

Product	Size	Cat.#
ReliaPrep™ miRNA Cell and Tissue Miniprep System	10 preps	Z6210
	50 preps	Z6211
	250 preps	Z6212

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