Product Application

Isolating RNA from Cotton Leaf Tissue Using the ReliaPrep™ RNA Tissue Miniprep System

Isolate high quality, amplifiable RNA from cotton plant leaves using the ReliaPrep™ RNA Tissue Miniprep System.

Kit: ReliaPrep™ RNA Tissue Miniprep System (Cat. # Z6111)

Sample Type(s): Cotton leaf tissue

Input: Up to 20mg

Materials Required:

- ReliaPrep™ RNA Tissue Miniprep System (Cat. # Z6111)
- 95% Ethanol
- Isopropanol
- Bead-beating device
- Homogenization steel bead
- 2.0ml screw-top tubes
- Bead-beating device (e.g., MP Biomedicals FastPrep®-24 Instrument)
- Microcentrifuge

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

Further information can be found in Technical Manual #TM394, available at: www.promega.com/protocols

OR For further information, please contact **techserv@promega.com**

Protocol:

1. Using a 5mm punch, place desired number of punches (up to 20mg) into a 2ml screw-top tube.
2. Prepare LBA Buffer solution by combining 20µl of 1-Thioglycerol per milliliter of LBA Buffer. Chill before using.
3. To each sample add:
    a. 500µl of prepared chilled 1-Thioglycerol/LBA Buffer solution.
    b. 1 homogenization bead
4. Using the bead-beating device, homogenize samples for desired time (e.g., MP Biomedicals FastPrep®-24 Instrument at 6m/s for 40 seconds).
5. Centrifuge samples at maximum speed for 3 minutes.
6. Add 170µl of Isopropanol to the samples and vortex.
7. Place once Minicolumn into a collection tube, per sample, and transfer the lysate to the column, being careful to avoid debris.
8. Centrifuge samples at maximum speed for 1 minute.
9. Remove the Minicolumn and discard the liquid, replace the column in the collection tube and add 500µl of RNA Wash Solution (with ethanol) to the column.
10. Centrifuge sample at maximum speed for 30 seconds. Remove the Minicolumn and discard the liquid, replace the column in the collection tube.
11. Prepare the DNase I incubation Mix by combining the following reagents in order:
    a. 24µl Yellow Core Buffer

b. 3µl $MnCl_2$, 0.09M
c. 3µl DNase I

12. Apply 30µl of DNase I incubation mix to the membrane of each Minicolumn and incubate for 15 minutes at room temperature.
13. Add 200µl of Column Wash Solution (with ethanol) to the Minicolumn and centrifuge at maximum speed for 15 seconds.
14. Add 500µl of RNA wash Solution to column and centrifuge at maximum speed for 30 seconds.
15. Place the Minicolumn into new collection tube and add 300µl of RNA Wash Solution (with ethanol) and centrifuge at maximum speed for 2 minutes.
16. Transfer the Minicolumn to an elution tube and add 30µl of Nuclease-Free Water to the column.
17. Centrifuge at maximum speed for 1 minute.
18. Eluates are ready for use in downstream applications.

Results:

Table 1. Cotton leaf RNA concentrations, yields, and purity based on quantitation using the QuantiFluor® RNA System (Cat. # E3310) and the NanoDrop One. N=3.

Sample	NanoDrop One			QuantiFluor® RNA	
	ng/µl	A_{260}/A_{280}	A_{260}/A_{230}	ng/µl	Yield (µg)
Cotton Leaf	11.90	1.94	0.27	21.00	0.42

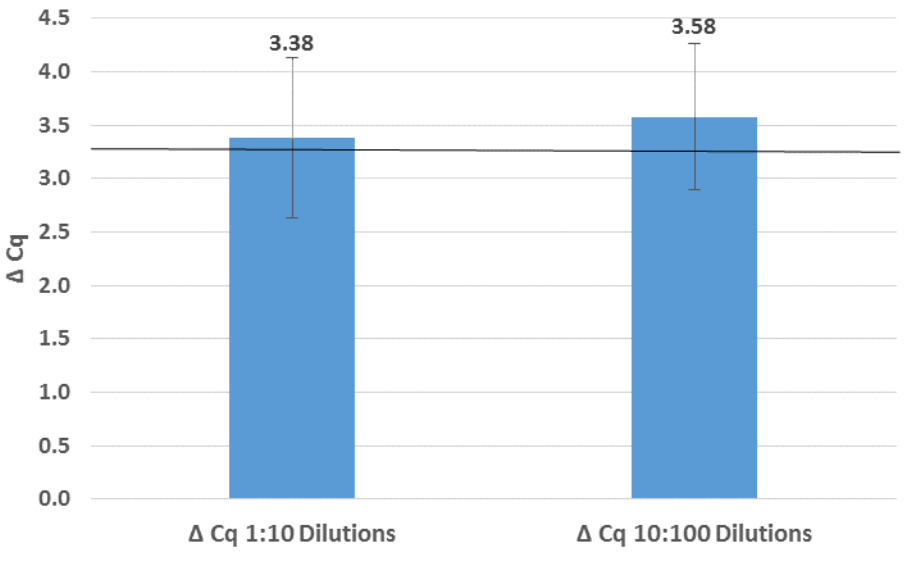

Figure 1. Inhibition analysis of purified cotton leaf RNA. RNA samples were serial diluted 1:10 and 10:100. For a sample diluted 10-fold, ΔCq values are expected to be 3.3. ΔCq values significantly less than 3.3 may indicate the presence of inhibitors. ΔCq values of RNA from cotton leaf samples indicate no inhibition of the serial diluted eluates. N=3.

