

Rice (*Oryza sativa*) Leaf RNA Purification

Isolate high quality, amplifiable RNA from rice leaf using the ReliaPrep™ System.

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

Analyses: GoTaq® RT-qPCR, QuantiFluor® quantitation

Sample Type(s): Rice (*Oryza sativa*) Leaf

Input: up to 40mg leaf tissue

Materials Required:

- ReliaPrep™ RNA Tissue Miniprep Kit (Z6111)
- Liquid Nitrogen
- Mortar and Pestle
- Isopropanol
- 95% Ethanol
- Tissue Homogenizer (e.g., Tissue-Tearor™ homogenizer)
- Microcentrifuge

This protocol was developed by Promega Applications Scientists. It is not intended to replace in-house validation.

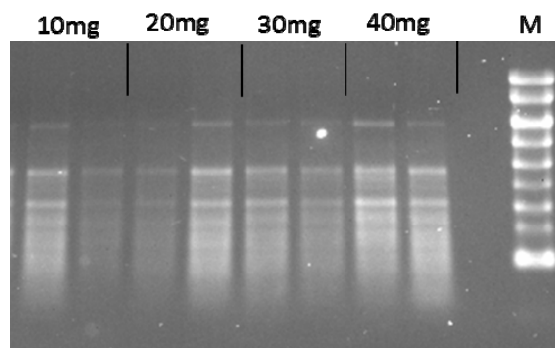
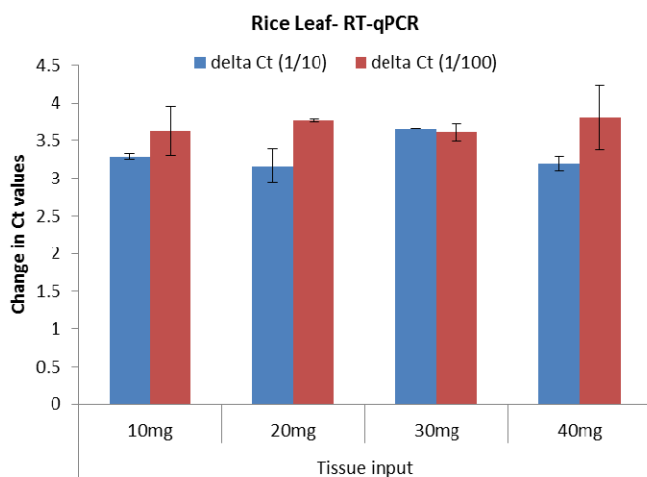
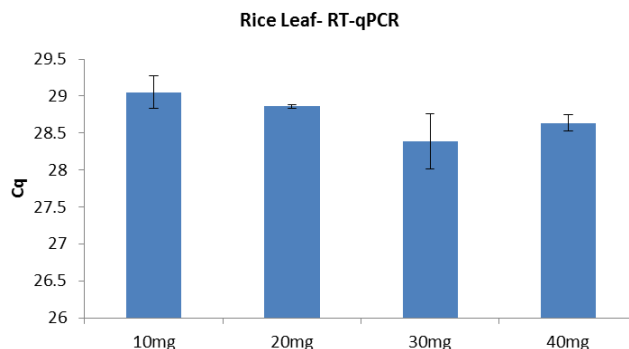
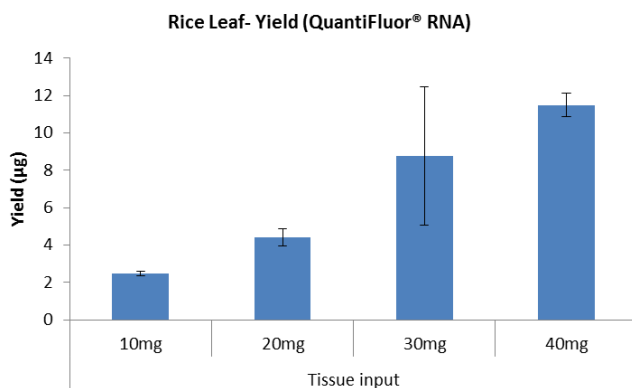
Further information can be found in Technical Manual #TM394, available at: www.promega.com/protocols

Protocol: (following the non-fibrous tissue protocol)

1. Prepare solutions as described in the technical manual (TM394).
2. Grind tissue sample material in liquid nitrogen using a mortar and pestle.
3. Add up to 40mg of ground sample to a 2ml tube.
4. Add 500µl of LBA + TG Buffer to the tube.
5. Homogenize samples with a small tissue homogenizer for 30-60 seconds.
6. Clear homogenates by centrifugation for 3 minutes at 14,000 x *g*, then transfer to a clean tube.
7. Add 170µl of isopropanol. Mix by vortexing for 5 seconds.

Proceed with the protocol in the technical manual (TM394) to purify the RNA using the ReliaPrep™ minicolumn.

Results:



Top Left: Rice leaf RNA yields are based on quantitation using the QuantiFluor™ RNA System (n=3). **Top Right:** GoTaq® 1-Step RT-qPCR analysis of purified RNA with rice specific primers (TaqMan® primer and probe set from Life Technologies, Cat. #4351372) using 4µl RNA eluate per 20µl reaction. **Bottom Left:** Changes in Ct values of samples indicate no inhibition of serial diluted eluates. **Bottom Right:** Examples of RNA purified from the indicated tissue masses analyzed on a 1.0% agarose gel with 5µl RNA eluate per lane. M=RNA Markers (G3191).