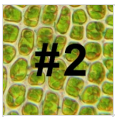

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RNA Isolation from Plant Tissue Protocol 2: McKenzie et al's Qiagen hybrid method

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1 Works for me dx.doi.org/10.17504/protocols.io.4q4gvyw

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ABSTRACT

Implemented by: Jim Leebens-Mack and Charlotte Carrigan

This protocol was developed by McKenzie et al.[2] to facilitate the isolation of RNA from woody plants rich in phenolics and polysaccharides, such as grapes (Vitaceae) and fruit bearing Rosaceae (apples, cherries and pears). The protocol is provided on Qiagen's website as an alternative method to be used in combination with their RNeasy Plant Minikit. We repeat the protocol here in the event that this protocol is removed from Qiagen's website or readers find it difficult to obtain the original publication.

This protocol is part of a collection of eighteen protocols used to isolate total RNA from plant tissue. (RNA Isolation from Plant Tissue Collection: https://www.protocols.io/view/rna-isolation-from-plant-tissue-439gyr6)

[2] McKenzie, D.J., McLean, M.A., Mukerji, S. & Green, M. Improved RNA extraction from woody plants for the detection of viral pathogens by reverse transcription-polymerase chain reaction. Plant Disease 81, 222-226 (1997).

journal.pone.0050226.s011.PDF

MATERIALS

NAME	CATALOG #	VENDOR
RNeasy Plant Mini Kit	74904	Qiagen

MATERIALS TEXT

Reagents

Lysis Buffer:

- 4 M guanidine isothiocyanate
- 0.2 M sodium acetate, pH 5.0
- 25 mM EDTA
- 2.5 % (w/v) PVP-40 (polyvinylpyrollidone, average molecular weight, 40,000)
- 1 % (v/v) β-mercaptoethanol (β-ME); add immediately before use

Other reagents:

- 20 % (w/v) sarkosyl
- Ethanol (96–100 %)

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

1 Grind sample (up to **50 mg**) in liquid nitrogen to a fine powder using a mortar and pestle.

Incomplete grinding of the starting material will lead to reduced RNA yields.

1.1 ⚠

Transfer the tissue powder and liquid nitrogen to an appropriately sized tube, and allow the liquid nitrogen to evaporate.

Do not allow the sample to thaw.

Continue immediately with step 2.

2 Add **600 µl** lysis buffer to a maximum of **50 mg** of tissue powder. Vortex vigorously.

3 Add **60 µl** of 20% sarkosyl.

For samples with a high starch content, incubation at elevated temperatures should be omitted to prevent swelling of the starting material.

3.1 Incubate at **70 °C** in a water bath or heating block for **00:10:00** with vigorous shaking or intermittent vortexing.

4 Pipet the lysate directly onto a QIAshredder Spin Column (lilac-colored column, supplied in the RNeasy Plant Mini Kit) placed in a 2 ml collection tube.

4.1 Centrifuge for **00:02:00** at maximum speed (>**14000 x g** [rcf]).

5 Carefully transfer the supernatant of the flow-through fraction to a new microcentrifuge tube without disturbing the cell-debris pellet in the collection tube.

It may be necessary to cut the end off the pipet tip in order to pipet the lysate onto the QIAshredder Spin Column. Centrifugation through the QIAshredder Spin Column removes cell debris and simultaneously homogenizes the lysate. While most of the celldebris is retained on the QIAshredder Spin Column, a very small amount of cell debris will pass through and form a pellet in the collection tube. Be careful not to disturb this pellet while transferring the lysate to a new microcentrifuge tube.

6 Add 0.5 volumes (usually **300 µl**) ethanol (96–100%), and mix well by pipetting.

If some lysate is lost during homogenization (step 4), reduce volume of ethanol proportionally. A precipitate may form after the addition of ethanol, but this will not affect the RNeasy procedure.

7 Continue with step 6 of the RNeasy Mini Protocol for Isolation of Total RNA from Plant Cells and Tissues and Filamentous Fungi in the RNeasy Mini Handbook.

RNA Isolation from Plant Tissue Protocol 6: pBIOZOL and Qiagen RNeasy Plant Mini Kit Method
by Eric Carpenter

PREVIEW RUN

7.1 Grind tissue to a powder in liquid nitrogen.

7.2 Add **1.3 ml** of cold (**4 °C**) pBIOZOL reagent for up to **100 mg** of frozen ground tissue.

7.3 Mix by briefly vortexing or flicking the bottom of the tube until the sample is thoroughly re-suspended.

7.4 Incubate the tube for **00:05:00** at **Room temperature**.

Lay the tube down horizontally to maximize surface area during RNA extraction.

7.5 Centrifuge for **00:10:00** at **12000 x g** in a microcentrifuge at **Room temperature**.

7.6 Transfer the supernatant to a new **1.5 ml** RNase-free tube.

7.7 Add **100 µl** of **5 Molarity (M)** NaCl and **300 µl** chloroform.

7.8 Vortex vigorously.

7.9 Centrifuge at **12000 x g** for **00:10:00** .

7.10 Transfer the top aqueous phase to a new 1.5 ml RNase-free tube.

7.11 Add an equal volume of 5:1 acid phenol:chloroform to the tube.

7.12 Vortex the tube until the phases mix and appear cloudy.

7.13 Incubate at **20 °C** for **00:05:00** .

7.14 Centrifuge at **12000 x g** for **00:10:00** .

7.15 Transfer the top aqueous phase to a new 1.5 ml RNase-free tube.

7.16 Add to the aqueous phase equal volume of 24:1 chloroform:isoamyl alcohol.

7.17 Vortex the tube until the phases mix and appear cloudy.

7.18 Then incubate at **Room temperature** for **00:05:00** .

7.19 Centrifuge at **12000 x g** for **00:10:00** .

7.20 Transfer the top aqueous phase to a new 1.5 ml RNase-free tube.

7.21 Add 1/2 volume of 100 % ethanol.

7.22 Pour the contents of the tube into a Qiagen mini RNA spin column (pink), until the column is almost filled with liquid.

7.23 Cap the tube.

7.24 Centrifuge at **12000 x g** for **00:00:15** .

The column should be empty at the end of this spin.

7.25 Discard the flow-through from the collection tube.

7.26 Repeat the previous two steps with the same mini RNA spin column, until all of the liquid in the tube(s) has been passed through the column.

The nucleic acid is now bound to the silica membrane in the spin column.

7.27 Apply **700 µl** of solution RW1 to the spin column.

7.28 Cap the tube.

7.29 Centrifuge at **12000 x g** for **00:00:15** .

The column should be empty at the end of this spin.

7.30 Discard the flow-through from the collection tube.

7.31 Apply **500 µl** of solution RPE to the spin column.

7.32 Cap the tube

7.33 Centrifuge at 12000 x g for 00:00:15 .

The column should be empty at the end of this spin.

7.34 Discard the flow-through.

7.35 Repeat previous two steps one time. go to step #33

7.36 Spin at maximum speed for 00:02:00 to remove remaining liquid from the silica membrane.

7.37 Transfer the spin column to a new 1.5 ml conical bottom microcentrifuge tube.

7.38 Add 30 µl – 50 µl of RNase-free water to the column.

7.39 Then let tube incubate at 20 °C for 00:03:00 .

7.40 Spin at maximum speed for 00:01:00 to collect RNA solution.