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Protocol: RNeasy Plant Mini Kit for RNA Extraction

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Gonzalo Villarino[1]

[1]Cornell University

Puregene

Gonzalo Villarino
Cornell University

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We use this protocol and it's working

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Abstract

This protocol describes the RNA extraction process from cannabis flowers using the Qiagen RNeasy Plant Mini Kit. Given the lack of a standardized RNA extraction method for cannabis, this protocol provides optimized steps to ensure high RNA yield and integrity by minimizing degradation. The workflow includes sample collection in the greenhouse (GH), flash-freezing in liquid nitrogen (LN2), mechanical disruption, and column-based RNA purification. Pre-warmed RNase-free water at 37°C is used to maximize RNA elution efficiency.

Attachments

PDF

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735KB

Guidelines

- Work quickly to prevent RNA degradation.
- Keep all tools and surfaces RNase-free by cleaning them with RNaseZap or 70% ethanol.
- Use fresh gloves and sterile tools for each sample.
- Flash-freeze all plant material immediately after liquid nitrogen or dry ice collection.
- Keep sample tubes and reagents cold during processing unless otherwise stated.
- Ensure ethanol (96-100%) is freshly prepared before use.

Safety warnings

- Do not allow tissue to thaw at any point before lysis.
- Buffer RLT and RW1 contain guanidine salts—avoid using with bleach or acid-based disinfectants.
- Ethanol is flammable—keep away from flames and heat sources.
- Wear protective equipment (gloves, lab coat, goggles) when handling β-mercaptoethanol.
- Always use an RNA-dedicated pipette and work in an RNase-free area.

Before start

Equipment:

- Portable analytical balance
- Liquid nitrogen (LN2) dewar
- Mortar & pestle (pre-chilled)
- Microcentrifuge (capable of ≥10,000 rpm)
- Pipettes (2 µL – 1000 µL) and RNase-free pipette tips
- Heat block set at 37°C (for pre-warming elution buffer)

Reagents & Consumables:

- Qiagen RNeasy Plant Mini Kit
- RNase-free water
- Buffer RLT (with β-mercaptoethanol, if needed)
- Buffer RW1
- Buffer RPE (ensure ethanol is added before use)
- Ethanol (96–100%)
- Sterile scissors & tweezers
- RNase-free 50 mL Falcon tubes (for sample freezing)
- RNase-free 2 mL & 1.5 mL microcentrifuge tubes
- QIAshredder spin columns (lilac)
- RNeasy spin columns (pink)

Preparation Before Going to the Greenhouse (GH)

1 Set up a portable **analytical balance** in the GH

2 Label **one tube** per cultivar. Use 50 mL Falcon tubes

3 Bring **RNase-free** gloves and sterile tools (scissors, tweezers).

Go to the greenhouse and collect fresh cannabis flowers

4 Cut fresh flowers using **sterile scissors** (one sample at a time)

5 Place the **weighed ~100 mg** sample into a labeled 50 mL Falcon tube. Poke some holes in lid of Falcon tube.

6 **Flash-freeze** the plant material (in 50 mL Falcon tube) immediately in liquid nitrogen

Go to lab

1m

7 Grind the frozen material **thoroughly** with a **mortar and pestle** while keeping it in liquid nitrogen.

1m

8 Transfer the finely powdered material to an **RNase-free, pre-chilled 2 mL** microcentrifuge tube (not supplied)

RNA Extraction Protocol

9 Add **450 µL Buffer RLT** to the 2 mL with the plant material. **Vortex** (1 minute) **vigorously** for complete lysis. Make sure the sample **looks homogenous** (no visible chunks or fibrous material)

10 Transfer **ALL** the lysate **(~450 µL)** from the 2 mL tube to a QIAshredder spin column (lilac) placed in a 2 ml collection tube. Centrifuge for **2 min at full speed.**

11 Transfer the supernatant **(~400 µL)** of the flow-through to a new **RNase-free 1.5 mL** microcentrifuge tube (not supplied) without disturbing the cell debris pellet in the collection tube. Use only this supernatant in subsequent steps. Discard only the QIAshredder spin column.

12 Add **~200 µL** (0.5 volume of 100% ethanol) to the cleared lysate and mix immediately by **pipetting (DO NOT VORTEX)**. Do not centrifuge. Proceed immediately to step 13.

13 Transfer the sample **(~600 µl)**, including any precipitate that may have formed, to an RNeasy spin column (pink) placed in a 2 ml collection tube (supplied).

14 Close the lid and centrifuge for **15 seconds** at ≥8000 x g (≥10,000 rpm). Discard the flow-through

15 Add **700 µl** Buffer **RW1** to the RNeasy spin column. Close the lid and centrifuge for **15 seconds** at ≥8000 x g (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.

16 Add **500 µl** Buffer **RPE** to the RNeasy spin column. Close the lid and centrifuge for **15 seconds** at ≥8000 x g (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.

17 Add **500 µl** Buffer **RPE** to the RNeasy spin column. Close the lid and centrifuge for **2 min** at ≥8000 x g (≥10,000 rpm) to wash the spin column membrane.

18 Place the RNeasy spin column in a **new 2 ml collection tube** (supplied), and discard the old collection tube with the flow-through. Close the lid and centrifuge at full speed for **1 min.**

19 Pre-warming the **RNase-free water** to **37°C** in heat block

20 Place the RNeasy spin column in a **new 1.5 ml collection tube** (supplied). Add **30 µl RNAse-free water** directly to the spin column membrane. **Let it sit for 1 minute** at room temperature. Close the lid. Centrifuge for **1 min** at ≥8000 x g (≥10,000 rpm) to elute the RNA.

Protocol references

Qiagen RNeasy Plant Mini Kit Handbook

- Qiagen (2023). RNeasy Mini Handbook. Qiagen GmbH, Hilden, Germany.
- Available Online