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RNA TRIzol isolation of *Datura* spp.

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Abstract

We present the TRIzol protocol for RNA extraction. We employed leaf tissue from vegetative and reproductive plants from four solanaceous species of the genus *Datura; D. stramonium, D. pruinosa, D. inoxia and D. wrightii.*

Guidelines

Set flash-frozen tissue

Prepare reagents

Isolate and store RNA

Quantify and verify RNA quality and integrity

Materials

Biological material: Leaf tissue

Reagents: Chloroform:Isoamyl, saline solution, 75% ethanol, isopropyl alcohol, TRIzol reagent, DNAsa I, RNase free water.

Laboratory devices: Centrifuge, tissuelyser, vortex mixer, thermo-shaker for microtubes

Other lab supplies: mortar and pestle, 1.5 ml Eppendorf tube, steel beads

Before start

Make sure all your lab devices and supplies are sterilized

REAGENTS

1 **Reagents**

Chloroform:Isoamyl alcohol (24:1) 100ml
96 ml Chloroform
4 ml Isoamyl alcohol

Saline solution 500 ml
0.8M Sodium Citrate
1.2 M NaCl
Distilled water

75% ethanol 100 ml
75 ml of ethanol molecular biology grade (100%)
25 ml of Distilled water

Isopropyl alcohol (Isopropanol) molecular biology grade

TRIzol reagent (Invitrogen/Thermofisher)

DNAsa I

RNase free water

RNA ISOLATION

2 **Protocol** 5m
1. Grind tissue to a powder in liquid nitrogen with cold (-80°C 1 hr sterilized) mortar and pestle

3 In a 1.5 ml Eppendorf tube add 50-100 mg of ground tissue plus 1ml of TRIzol Reagent and 5 µl of DNAsa I. Mix by vortex 30 seconds. 2m

4 Add two sterilized steel beads and high-speed shake the tube in a Tissuelyser II (QIAGEN), at 30 Hz for 45 seconds. Repeat 2 more times 3m

5 Take out the beads and incubate the mixture at room temperature in the same Eppendorf tube for 5 min. 5m

6 Centrifuge the tube at 13,000 rpm for 15 min at 4°C. All of the insoluble matter should form a pellet at the bottom of the tube. 15m

7 Transfer the aqueous phase (upper) in a new 1.5 ml tube and add 250 µl of isoamyl alcohol. Mix by inversion. 2m

8 Centrifuge the tube at 13,000 rpm for 15 min at 4°C. 15m

9 Transfer the upper aqueous phase in a new 1.5 ml tube and add 250 µl of Saline solution and then 250 µl of isopropanol. 3m

10 Mix by inversion and incubate on ice for 10 min. 10m

11 Centrifuge at 13,000 rpm for 10 min at 4°C, and decant the supernatant, taking care not to lose the pellet. 10m

12 Add 1 ml 75% cooled ethanol to the pellet and centrifuge at 13,000 rpm for 10 min at 4°C and decant the supernatant 10m

13 Open the cap and air-dry the pellet. 5m

14 Add 30µl RNase-free water and heat at 55°C to resuspend the pellet carefully. 5m

15 Analyze (quantify concentration, quality, and integrity of the RNA) and store the tube at -80°C.

RNA QUALITY AND INTEGRITY

16 The integrity of each sample of RNA was first visualized by electrophoresis in 1.0% agarose stained with Ethidium Bromide (EtBr). The gel was run in TBE 0.5X buffer prepared in water treated with DEPC.

Then the quantity and quality of RNA samples were verified by UV absorbance in Nanodrop (Thermo Scientific) and by fluorescence measurements with Qubit 3.0 (Invitrogen) using 2ul of each sample of RNA .