



ZYMO RESEARCH

Modified CTAB Protocol

- For RNA isolation from polysaccharide-rich and/or phenolics-rich samples, including plant tissues (Pinus, Geranium...)
- For use in conjunction with **Direct-zol™ RNA** kits, **Quick-RNA™ Plant Miniprep**, or **RNA Clean & Concentrator™** kits

Ver.1.0.1

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(I) Buffer Preparation

1. Prepare **Modified CTAB Extraction Buffer** using the following components and autoclave after preparation:

2% CTAB
2% PVP40
25 mM EDTA
100 mM Tris, pH 8
2 M NaCl
0.5 g/L spermidine

2. Prepare **Chloroform-Isoamylalcohol Mixture**:

Chloroform : Isoamyl Alcohol = 24 : 1

(II) Purification

- ✓ Before extraction, supplement **Modified CTAB Extraction Buffer** with 3 % (v/v) β -mercaptoethanol.
1. For each sample, pre-heat 1.2 mL **Modified CTAB Extraction Buffer** (3 % (v/v) β -mercaptoethanol added) in a 2 mL Eppendorf tube at 65°C in a water bath.
 2. Homogenize 250 mg of leaf material in liquid nitrogen using a mortar and pestle. Transfer homogenized leaf material to pre-heated **Modified CTAB Extraction Buffer** (step 1).
 3. Vortex immediately for 1 minute then place in 65°C water bath. Keep in water bath for at least 30 minutes and vortex once every 5 minutes.
 4. Centrifuge at maximum speed (e.g., 16,000 x g) for 10 minutes and transfer supernatant to a new 2 mL Eppendorf tube.
 5. Add an equal volume (1:1) of **Chloroform-Isoamylalcohol Mixture**. Vortex for 30 seconds and centrifuge at maximum speed for 15 minutes at 4 °C.
 6. Transfer the aqueous phase to a new 2 mL Eppendorf tube without disrupting the white interphase.
 7. Repeat steps 5 and 6.
 8. Add an equal volume (1:1) of ethanol (95-100%) to the aqueous phase and mix well.
 9. Continue to the **Direct-zol™ RNA** kit protocol (e.g., [Direct-zol RNA Miniprep](#), RNA Purification, step 2), the **Quick-RNA™ Plant Miniprep** protocol (Total RNA Purification, step 6), or the **RNA Clean & Concentrator™** kit protocol (e.g., [RNA Clean & Concentrator-25](#), Total RNA Clean-Up, step 3) by loading the mixture into the provided **Zymo-Spin column**. Perform remaining steps at room temperature.