



(DP424) TRNzol Universal Reagent ——Plant

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Experiment Preparation

1. Plant Leaves; Mortar; Liquid nitrogen
2. Chloroform; Isopropanol; RNase-Free ddH₂O; 75% ethanol (Prepare with RNase-Free ddH₂O)
3. Pipette and matched RNase-free sterile tips (200 μ l, 1 ml); 1.5 ml centrifuge tubes (RNase-free)
4. Vortex oscillator; Refrigerated centrifuge



Step 1



Fully grind the tissues in liquid nitrogen.



Add 1 ml TRNzol Universal for every 50-100 mg tissue and mix well quickly.

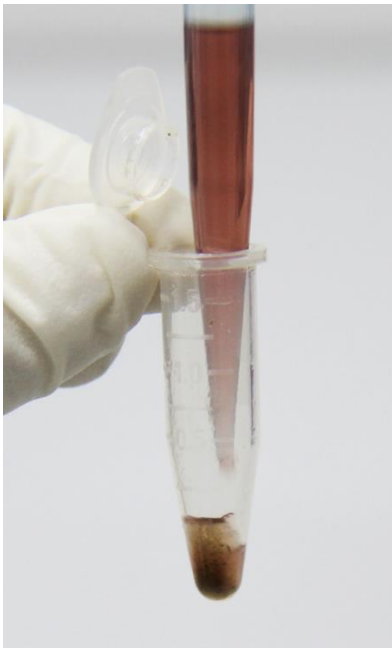
Note: To ensure that the sample does not thaw or degrade, Buffer RZ should be prepared first, and add the ground sample directly to the Buffer RZ. The sample volume should not exceed one-tenth of the RZ volume.

Step 2



Place the homogenate samples in 15-30°C for 5 min to make the nucleic acid protein complex completely separate. The color of the sample will become slightly darker at this step.

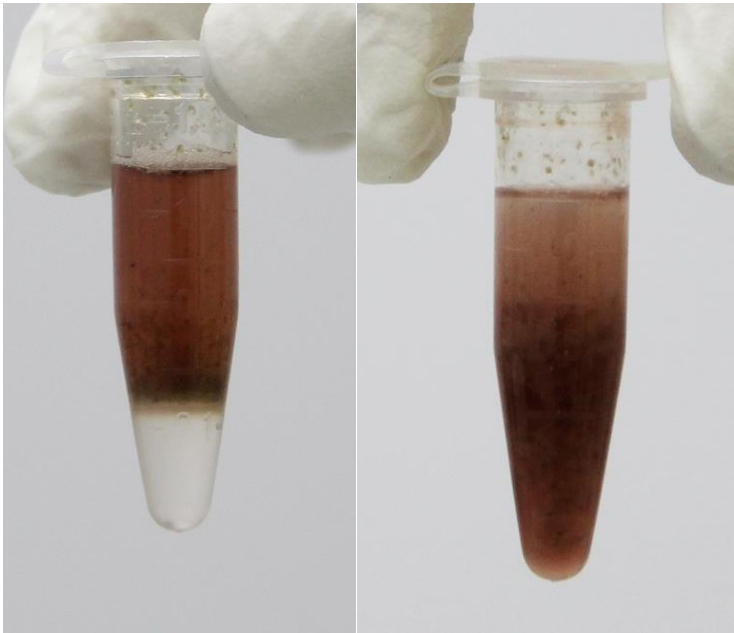
Step 3 (Optional)



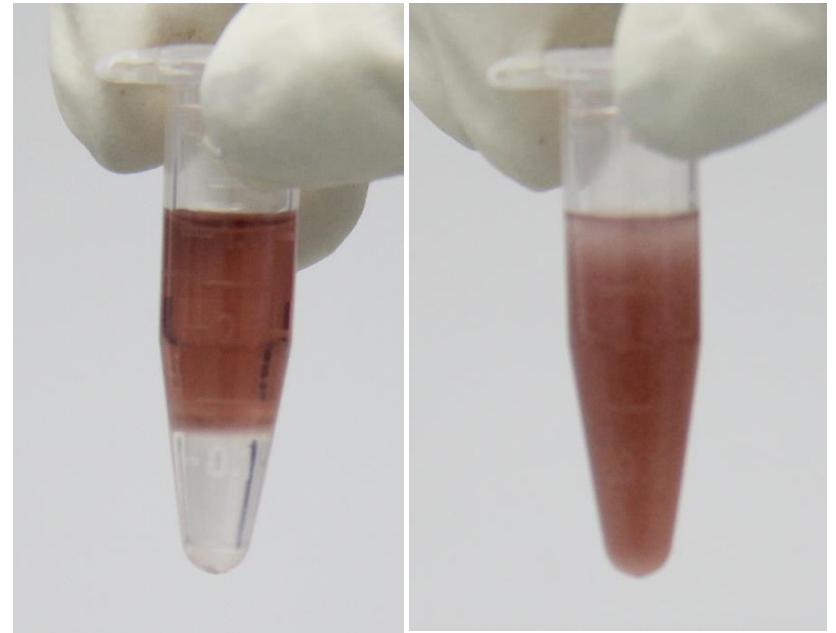
Centrifuge at 12,000 rpm (~13,400 g) at 4°C for 5 min, transfer the supernatant into a new RNase-free centrifuge tube.

Note: If the sample contains more protein, fat, polysaccharide or muscle parts, etc., this step can be included. The precipitates obtained by centrifugation include extracellular membrane, polysaccharide, while high molecular weight DNA and RNA in the supernatant solution.

Step 4



Not applied the centrifugation in step 3

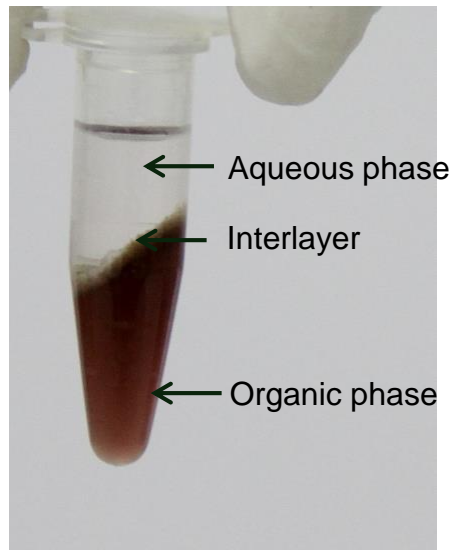


Applied the centrifugation in step 3

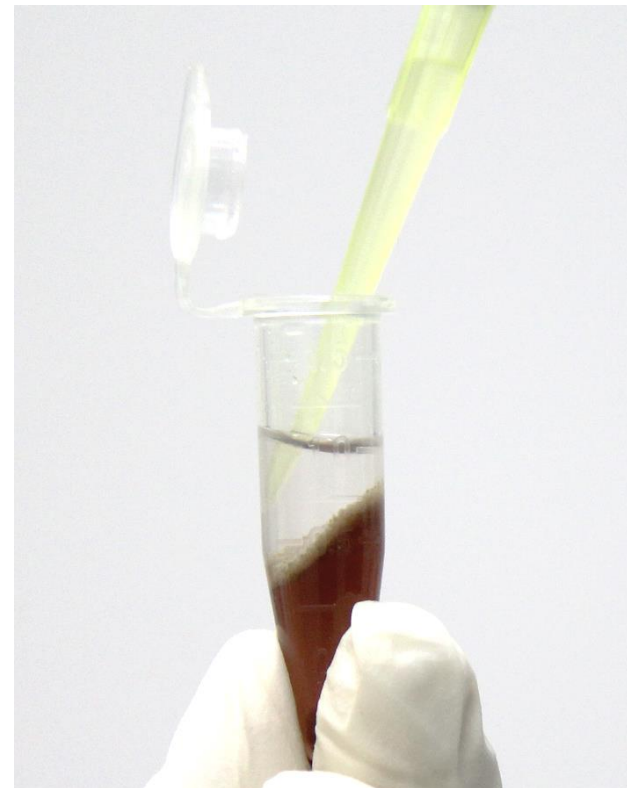
Add 200 μ l chloroform and vortex vigorously for 15 sec. Then place at room temperature for 3 min.

Step 5

The following steps are performed without the centrifugation in Step 3



Centrifuge at 12,000 rpm ($\sim 13,400 \times g$) at 4°C for 10 min.



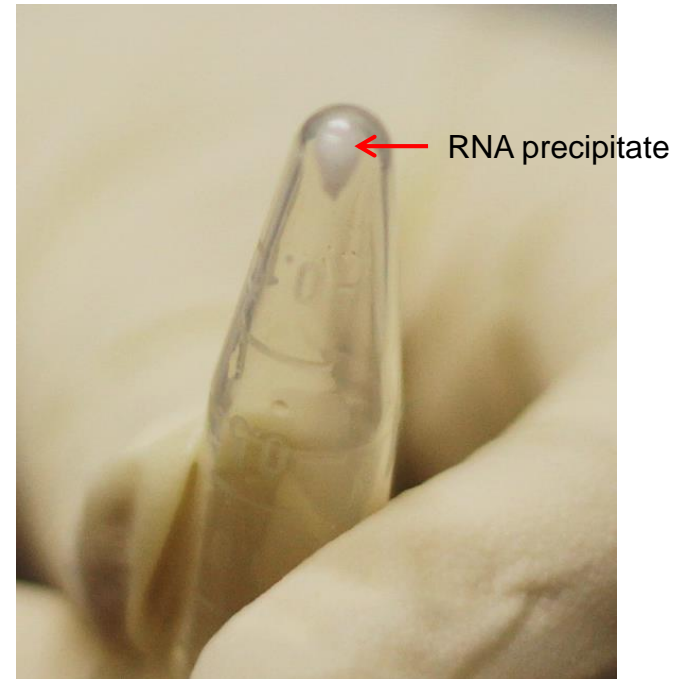
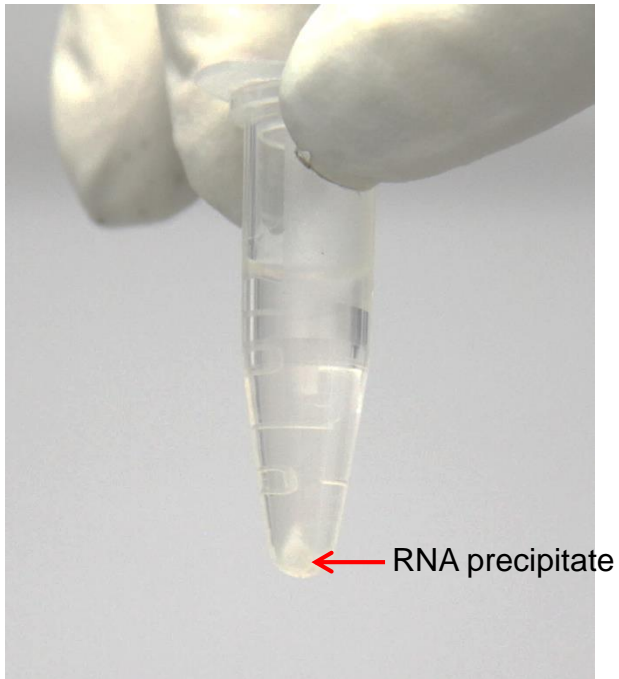
Transfer the water phase to a new tube for the next step. The volume of the water phase is approximately 50% of that of the TRNzol Universal used.

Step 6



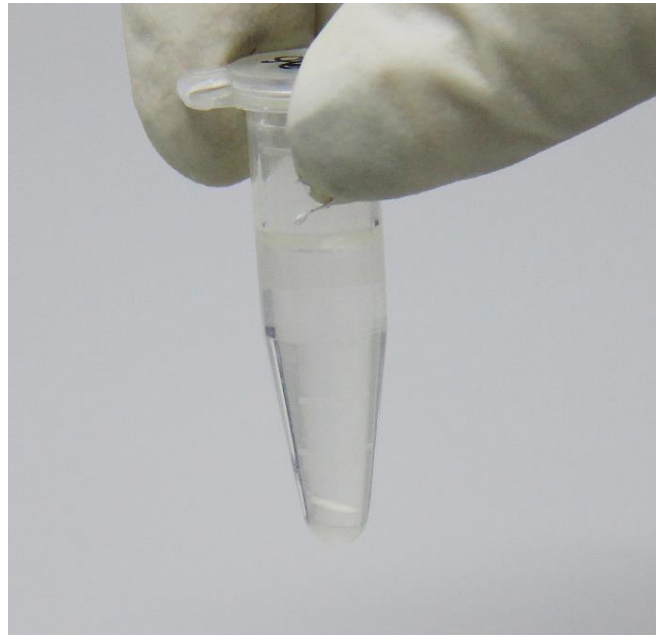
Add the same volume of isopropanol in the obtained water solution, mix well and place at room temperature for 20-30 min.

Step 7



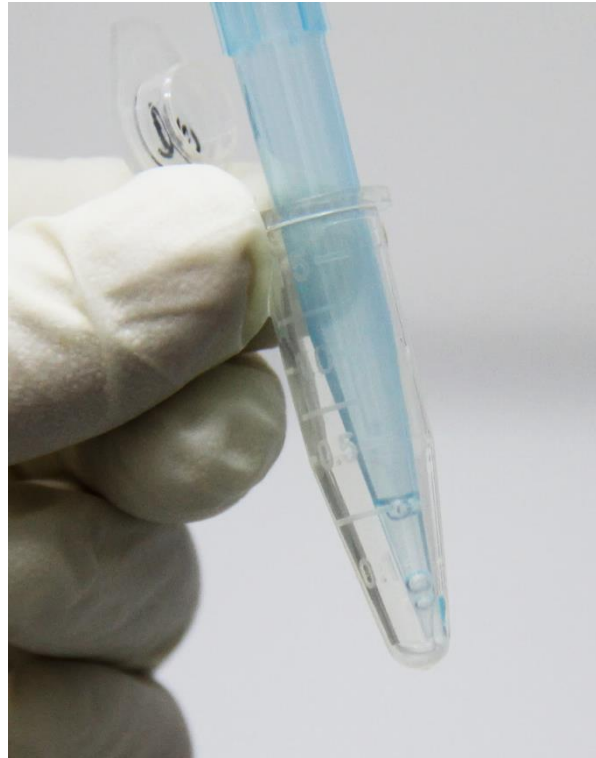
Centrifuge at 12,000 rpm ($\sim 13,400\times g$) at 4°C for 10 min, and remove the supernatant. RNA precipitation is often invisible before centrifugation, and colloidal precipitation will be formed on the side and bottom of the tube after centrifugation.

Step 8



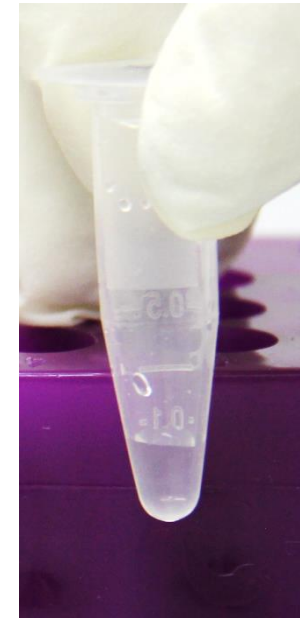
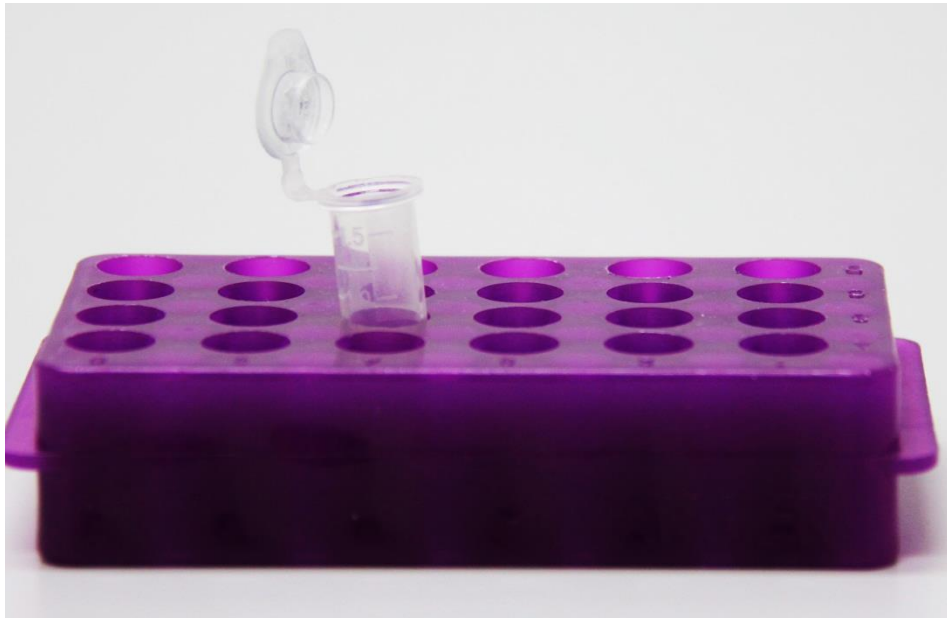
Add 1 ml 75% ethanol (prepare with RNase-free ddH₂O) to wash the precipitation. Wash with at least 1 ml 75% ethanol for every 1 ml TRNzol Universal.

Step 9



Centrifuge at 10,000 rpm ($\sim 9,391\times g$) at 4°C for 5 min, and discard the supernatant. Be careful not to pour out the precipitate. Centrifuge the remaining small amount of liquid for a short time, and then carefully remove the liquid without touching the precipitate.

Step 10



Dry at room temperature for 2-3 min (Do not over-dry, or the RNA will be difficult to dissolve). According to the experiment needs, add 30-100 μ l RNase-free ddH₂O, pipette up and down repeatedly to fully dissolve RNA.