(DP419) RNAsimple Total RNA Kit ——Plant

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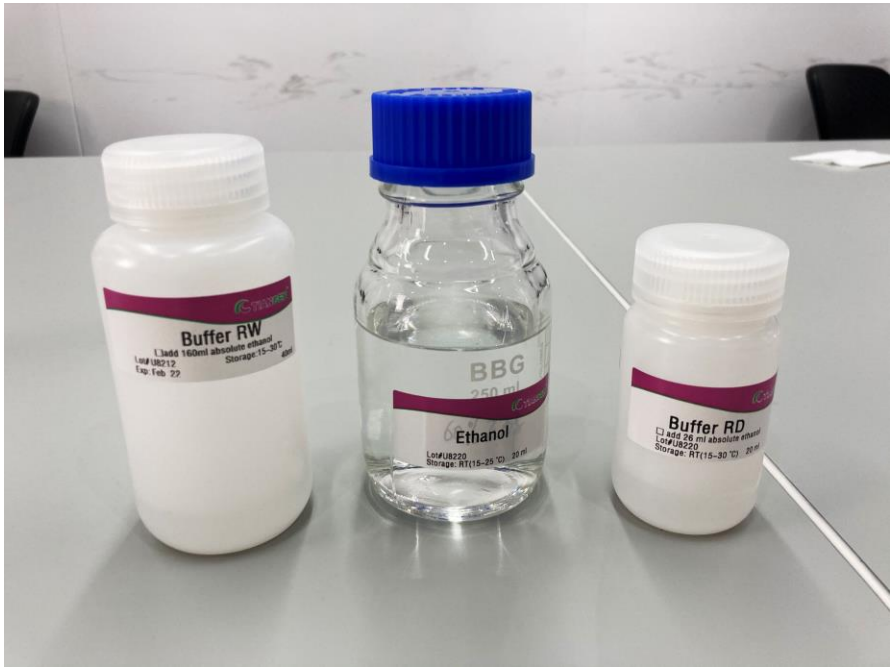
Experiment Preparation

1. Plant leaves; Mortar; Liquid nitrogen
2. 96-100% ethanol; Chloroform
3. Pipette and matched RNase-Free tips (200 μl, 1 ml); 1.5 ml & 2 ml centrifuge tubes (RNase-free)
4. Vortex; Refrigerated centrifuge

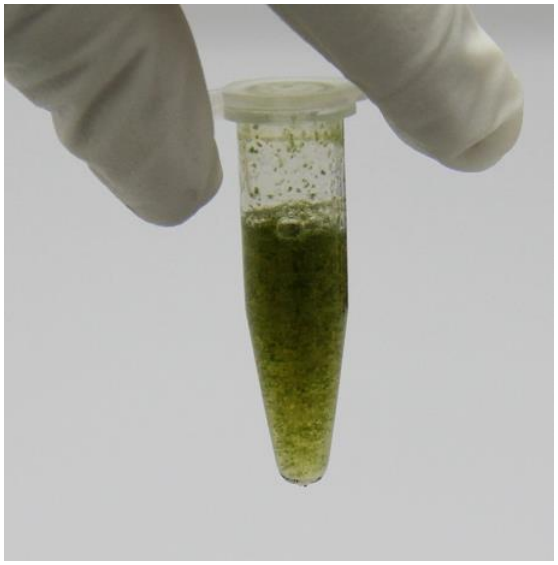
Experiment Preparation - Kit Preparation

Please add 96-100% ethanol in Buffer RW and RD before use according to the volume indicated on the label of the bottle.



Step 1

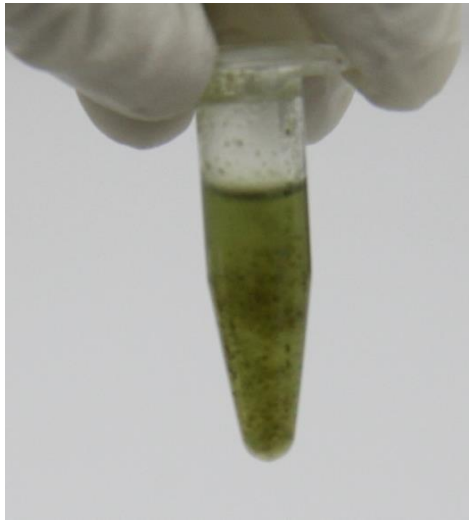
Fully grind the tissues in liquid nitrogen.



Add 1 ml Buffer RZ for every 50-100 mg tissue and mix well quickly.

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

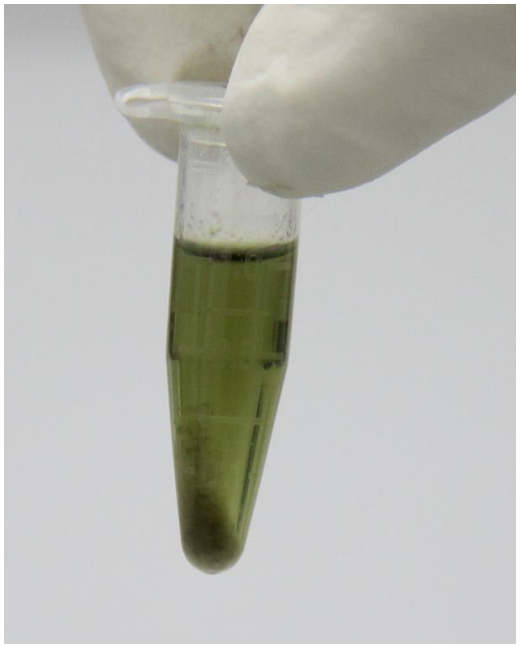
Step 2



Place the homogenate samples at 15-30 ℃ for 5 min, to make the nucleic acid protein complex completely separate.

The color of the sample will become slightly darker.

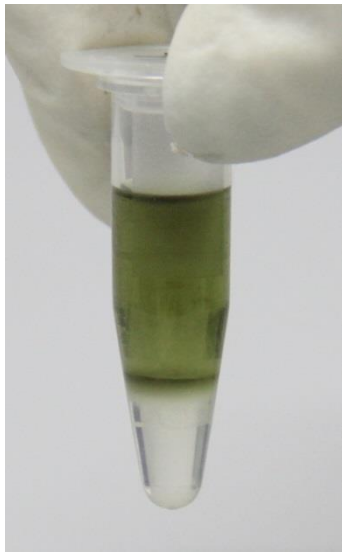
Step 3 (Optional)



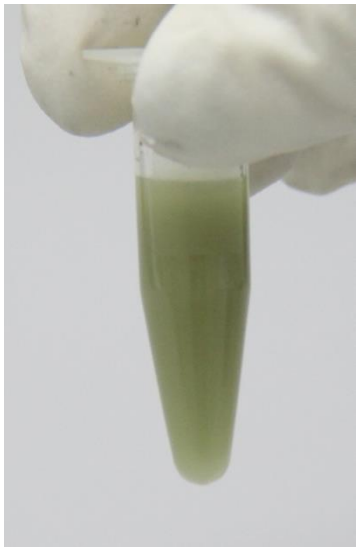
Centrifuge at 12,000 rpm (~13,400 g) at 4℃ for 5 min, transfer the supernatant to a new RNase-free centrifuge tube.

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

Step 4

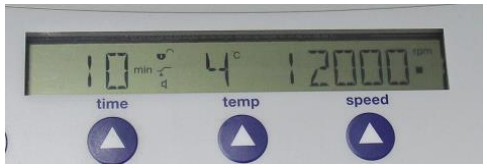


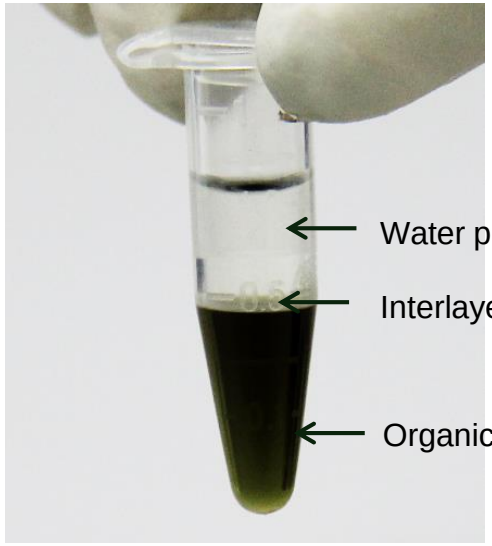
Add 200 μl chloroform.



Vortex vigorously for 15 sec and place at room temperature for 3 min.

Step 5

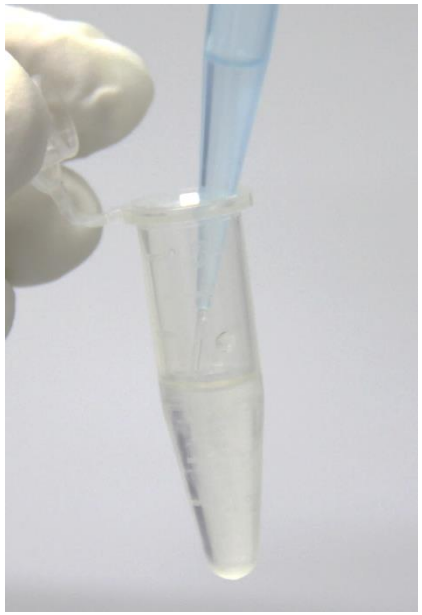




Centrifuge at 12,000 rpm (~13,400×g) 4℃ for 10 min.

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 Buffer RZ used.

Step 6



Slowly add 0.5 × volume of 96-100% ethanol and mix well (precipitation may occur).

Transfer the solution and precipitate to Spin Column CR3. Centrifuge at 12,000 rpm (~13,400×g) at 4℃ for 30 sec. Discard the waste liquid in the collection tube.

If all solutions and mixtures cannot be added to Spin Column CR3 at one time, please transfer them into Spin Column CR3 in two times.

Step 7

Add 500 μl Buffer RD into Spin Column CR3 (**make sure 96-100% ethanol has been added before use**). Centrifuge at 12,000 rpm (~13,400×g) and 4℃ for 30 sec, pour out the waste liquid in the collection tube and place the Spin Column CR3 back into the collection tube.

Step 8

Add 500 μl Buffer RW to Spin Column CR3 (**make sure 96-100% ethanol has been added before use**), place at room temperature for 2 min, and centrifuge at 12,000 rpm (~13,400×g) and 4℃ for 30 sec, pour out the waste liquid.

Step 9 Repeat step 8.

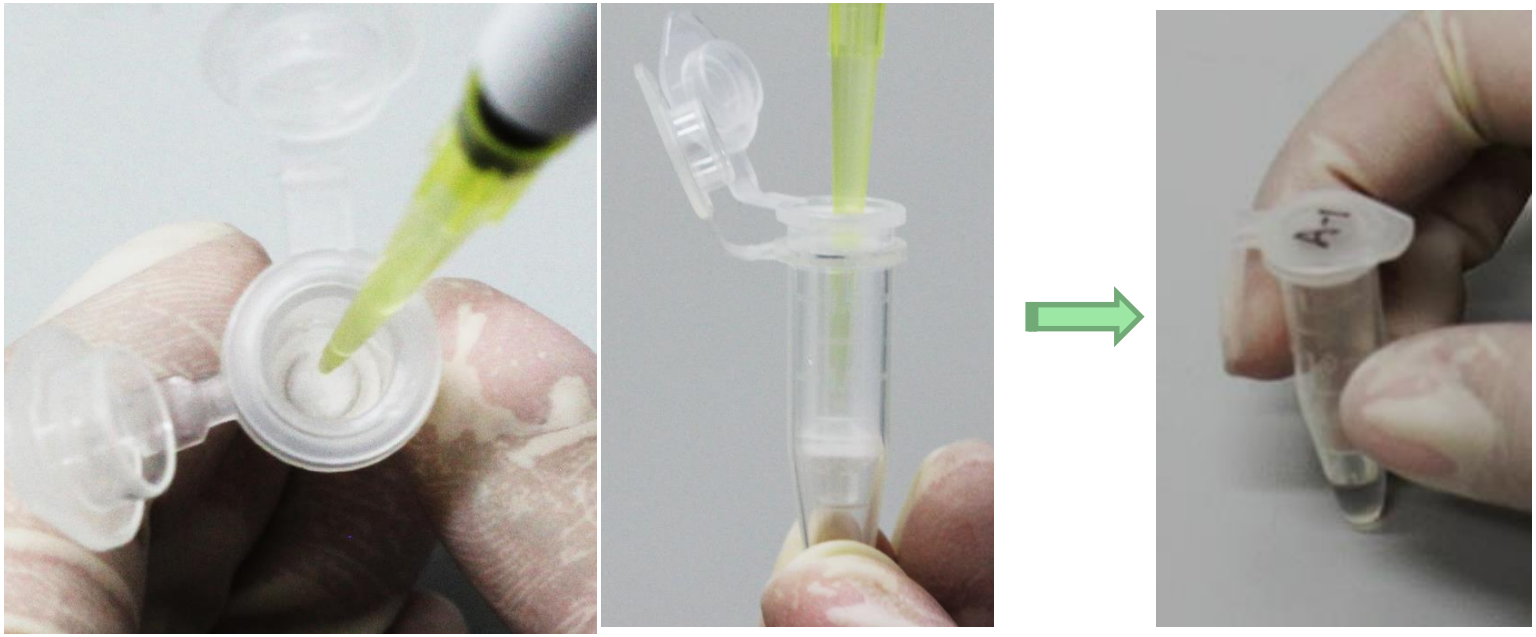
Step 10

Place the Spin Column into a new 2 ml collection tube. Centrifuge at 12,000 rpm (~13,400×g) for 2 min, and discard the waste liquid.

Place the Spin Column CR3 at room temperature for a moment, or place on the ultra-clean working table to ventilate for a moment to fully dry the membrane.

Note: Ethanol residues in Buffer PW can inhibit subsequent enzymatic reactions (restriction enzyme digestion, PCR, etc.) experiments.

Step 11



Transfer the Spin Column CR3 into the centrifuge tube provided with the kit, add 30–100 μl RNase-Free ddH_2O, place at room temperature for 2 minutes and centrifuge at 12,000 rpm (~13,400×g) at 4℃ for 2 min.

The volume of elution buffer should not be less than 30 μl. Too small volume affects the recovery efficiency. RNA should be kept at -70℃ to prevent degradation.