



# (DP320) DNAsecure Plant Kit- Plant

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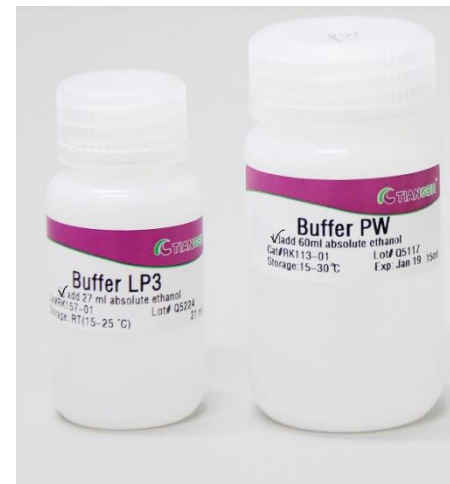
# Experiment Preparation

1. Plant Leaves
2. Mortar; Liquid nitrogen
3. Pipette and matched sterile tips (10  $\mu$ l, 200  $\mu$ l, 1 ml); 1.5 ml centrifuge tubes
4. 96-100% ethanol
5. Vortex oscillator; Dry bath/water bath; Centrifuge



# Experiment Preparation-Kit Preparation

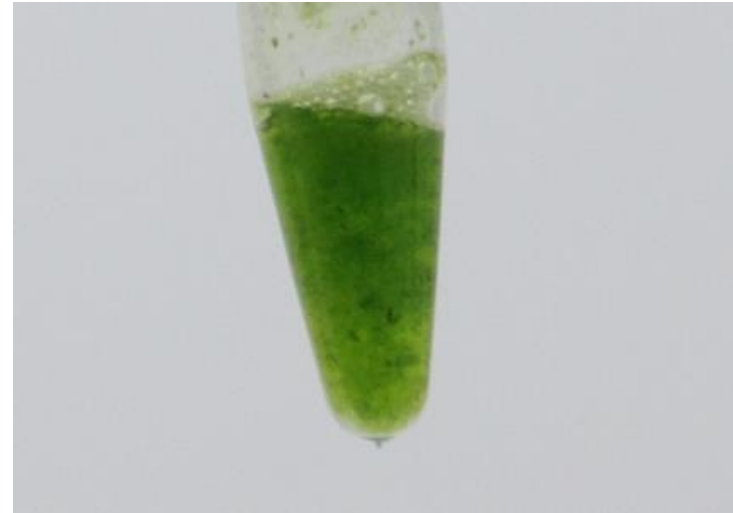
Please add 96-100% ethanol in Buffer PW and LP3 before use according to the volume indicated on the label of the bottle.



# Step 1



Add liquid nitrogen to 100 mg fresh plant tissue or 20 mg dry weight tissue, and fully grind the tissues.



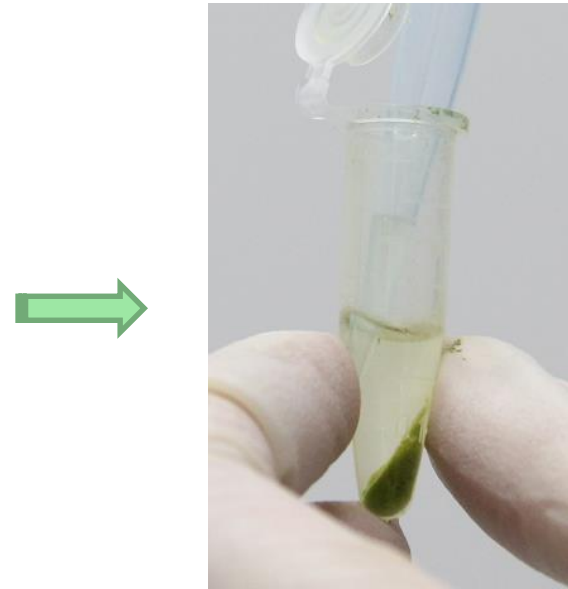
Add 400  $\mu$ l Buffer LP1 and 6  $\mu$ l RNase A (10 mg/ml). After vortex for 1 min, place at room temperature for 10 min.

## Step 2



Add 130  $\mu$ l Buffer LP2, mix well by vortex for 1min.

## Step 3



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

## Step 4



Add  $1.5 \times$  volume of Buffer LP3 (e.g. add 750  $\mu$ l Buffer LP3 to 500  $\mu$ l supernatant) (make sure 96-100% ethanol has been added before use).

Fully mix by vortex for 15 sec immediately, and there may be flocculent precipitate appeared.

## Step 5



Transfer the mixed liquid into Spin Column CB3.



Centrifuge at 12,000 rpm (~13,400×g) for 30 sec. Discard the waste liquid in the collection tube and place the adsorption column CB3 into the collection tube.

## Step 6



Add 500  $\mu$ l Buffer PW to Spin Column CB3 (make sure 96-100% ethanol has been added before use)



Centrifuge at 12,000 rpm ( $\sim$ 13,400 $\times$ g) for 30 sec, discard the waste liquid in the collection tube and place the Spin Column CB3 into the collection tube.

**Note:** If the column membrane appears green, add 500  $\mu$ l 96-100% ethanol to the Spin Column CB3, centrifuge at 12,000 rpm ( $\sim$ 13,400 $\times$ g) for 30 sec, discard the waste liquid, and place the Spin Column CB3 into the collection tube.

## Step 7 Repeat step 6.



## Step 8

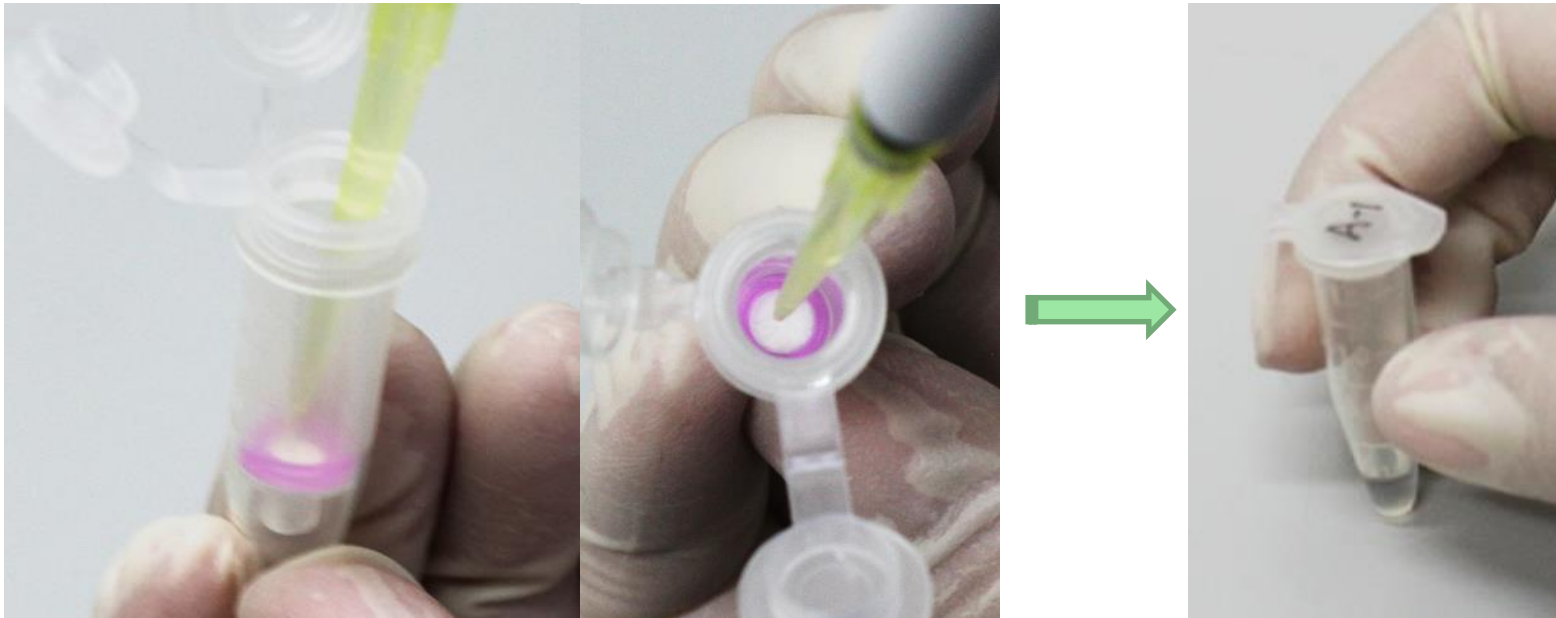


Centrifuge at 12,000 rpm ( $\sim 13,400\times g$ ) for 2 min, and discard the waste liquid.

Place the Spin Column CB3 at room temperature for 2 minutes to completely dry Buffer PW in the membrane.

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

## Step 9



Transfer the Spin Column CB3 into a 1.5 ml centrifuge tube, and add 50-200  $\mu$ l Buffer TE to the middle of the adsorption membrane. Place at room temperature for 2 min and centrifuge at 12,000 rpm ( $\sim 13,400 \times g$ ) for 2 min to collect the solution into the centrifuge tube.