

## Kit Content

	4rxn	50rxn	250rxn	
Spin Columns	4	50	250	pcs
Collection Tubes (2 ml)	12	150	750	pcs
Buffer RPL	2	25	125	ml
Buffer RB	0.5	6.3	31.5	ml
Buffer RW1 (concentrated)	3.36	42	105x2	ml
Buffer RW2 (concentrated)	0.68	8.4	42	ml
RNase-Free H <sub>2</sub> O	0.96	12	60	ml

## Kit Storage

Columns, buffers, solvents and consumables, please store at 15-25 °C.

## Kit Preparation

- 1. Prepare Buffer RB**  
Add 2.4 volume of 100% EtOH into Buffer RB and vortex thoroughly.  
After adding 100% EtOH, please tick the sticker on the bottle and close the cap tightly.
- 2. Prepare Buffer RW1**  
Add equal volume of 100% EtOH into Buffer RW1 (concentrated) to get Buffer RW1.  
After adding 100% EtOH, please check the sticker on the bottle and close the cap tightly.
- 3. Prepare Buffer RW2**  
Add 4 volume of 100% EtOH into Buffer RW2 (concentrated) to get Buffer RW2.  
After adding 100% EtOH, please check the sticker on the bottle and close the cap tightly.

## General Protocol

- Cut off 50 mg (up to 100 mg) of fresh or frozen plant tissue.
- Grind tissue sample thoroughly with liquid nitrogen by beads beater\*, tissue homogenizer or mortar & pestle. Transfer it into a liquid nitrogen pre-chilled micro-centrifuge tube.  
\*For grinding samples with beads beater. Please cut off 50 mg (up to 100 mg) of fresh or frozen plant tissue into 2ml micro-centrifuge tube. Add proper number (1-3) and proper dimension (3-7 mm) of stainless beads into the sample tube. Immerse tube(s) into liquid nitrogen for at least 2 mins before homogenizing. Homogenize for 30 sec at 30Hz (Do not let the tissue to thaw). Immerse sample tubes into liquid nitrogen and homogenize again, if there is still any large pieces.
- Add 450 µl of RPL Buffer (add 1%β-ME freshly) and vortex vigorously to lysis the sample. Incubate at room temperature for 5 min.
- Centrifuge at 11,000 x g for 2 min to spin down insoluble material and transfer 350 µl the clear supernatant to the 2ml Sample Tube.  
\*Avoid transferring any debris into the 2ml Sample Tube. Fill up with RPL Buffer if the clear supernatant is less than 350 µl.
- Add 350 µl Buffer RB, vortex for 30 sec then briefly spin down.
- Transfer all mixture to Spin Column (with 2ml Tube).
- Centrifuge at 11,000 x g for 1 min, and change a new collection tube.
- Add 700 µl Buffer RW1 into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
- (Optional) On column digest of DNA with DNase I (not provided).
- Add 700 µl Buffer RW1 into spin column, centrifuge at 11,000 x g for 1 min, discard the flow-through.
- Add 700 µl Buffer RW2 into spin column, centrifuge at 11,000 x g for 1 min.
- Change a new collection tube, centrifuge at 11,000 x g for 3 min.
- Place the spin column into 1.5 ml micro-centrifuge tube, add 30-100 µl RNase-Free H<sub>2</sub>O and incubate at 25°C (room temperature) for 2 min.
- Centrifuge at 11,000 x g for 1 min for elution.

**FOR RESEARCH USE ONLY**