

AccuPrep® Plant Genomic DNA Extraction Kit (K-3031)

I Before You Begin

- 1) Completely dissolve one vial of **Proteinase K** in **1,250 µl** of nuclease free water. For short term storage, dissolved Proteinase K should be stored at **4°C**. For long term storage, it is recommended to aliquot the enzyme into separate tubes and store at **-20°C**.
- 2) Completely dissolve one vial of **RNase A** in **600 µl** of nuclease-free water. For short term storage, dissolved RNase A should be stored at **4°C**. For long term storage, it is recommended to aliquot the enzyme into separate tubes and store at **-20°C**.
- 3) Add correct amount of **absolute ethanol** to **WA1 Buffer**.
- 4) Before starting extraction process, heat the **EA Buffer** at **56~60°C**.

II Experimental Protocol

- 1) Grind ~100 mg of plant to fine powder. Put the powder and **300 µl** of **PL Buffer** in a 1.5 ml or 2 ml tube. Powdered plants are efficient for lysis.
- 2) Add **20 µl** of **Proteinase K** and **10 µl** of **RNase A**. Mix thoroughly and make sure that you completely resuspend the sample to achieve maximum lysis efficiency.
- 3) Incubate the tube at **60°C** for **10 min**.
- 4) Add **100 µl** of **PC Buffer** and mix thoroughly.
- 5) Incubate for **5 min on ice**. This step precipitates detergent, proteins and polysaccharides.
- 6) After 5 min, centrifuge the tube at **13,000 rpm** for **5 min** then transfer the supernatant to a new tube.
(Option) If plant tissue remains in the supernatant, centrifuge once again.
- 7) Add **1.5 sample volumes** of **WA1 Buffer** to the cleared lysate and mix well using a vortex mixer or by pipetting. And then spin down for 10 sec to get the liquid clinging to the walls and lid of the tube.
- 8) Carefully transfer the lysate into the upper reservoir of the Binding column tube (fit in a collection tube) without wetting the rim.
- 9) Close the tube and centrifuge at **8,000 rpm** for **1 min**.
(Option) If the liquid has not completely passed the column following centrifugation, then centrifuge again until the liquid completely passes through.
- 10) Discard the solution from the collection tube and reuse the collection tube.
- 11) Repeat step 8~10 with remaining sample.
- 12) Add **500 µl** of **W2 Buffer** to the column, close the lid, and centrifuge at **8,000 rpm** for **1 min**.
- 13) Discard the solution from the collection tube and reuse the collection tube.

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- 14) Add **500 µl** of **W2 Buffer**, close the lid, and centrifuge at **8,000 rpm** for **1 min**.
- 15) Discard the solution from the collection tube and reuse the collection tube.
- 16) Centrifuge once more at **13,000 rpm** for **1 min** to completely remove ethanol, and check that there is no droplet clinging to the bottom of Binding column tube.
(Caution) Make sure that there is no droplet hanging from the bottom of the Binding column. Residual W2 Buffer left in the Binding column may cause problems in later applications.
- 17) Transfer the Binding column tube to 1.5 ml tube for elution, add **50~200 µl** of **EA Buffer** onto Binding column tube, and wait for at least 1 min.
(Option) We recommend letting stand for about 5 min to increase DNA yield. You can also increase yield by heating the EA Buffer at about 60°C before adding to the column.
- 18) Centrifuge at **8,000 rpm** for **1 min** to elute.

※ For more information, please visit www.bioneer.com and refer to the User's Guide of this kit.