AuPreP™ DNA easy Plant Maxi kit

Cat. # PLX68-163LTD

Isolation of genomic DNA from 1 g plant material.

Kit Contents:

PX1 Buffer	90 ml
PX2 Buffer	30 ml
PX3 Buffer	110 ml
RNase A	85 mg
WS Buffer	50 ml
Plant Genomic DNA Maxi Column	20
Shearing tube	20
15ml Collection tube	40
Protocol	1

Notes:

- All centrifugation should be done at room temperature with a swing-bucket centrifuge.
- Preheat a water bath to 65°C.
- Preheat TE or ddH_2O to 65°C for DNA elution.
- PX1 Buffer and PX3 Buffer may form a precipitate, warm at 65°C to redissolve.
- Add 850 µl of ddH_2O to the RNase A powder tube, vortex to dissolve and store at 4°C.

Protocol:

1. **Grind 1 g (or less) plant sample under liquid nitrogen to a fine powder and transfer to a new tube.**
   Do not allow the sample to thaw, and continue immediately to step 2.

2. **Add 4 ml of PX1 Buffer and 40 µl of RNase A solution (100 mg/ml) to the tissue powder and vortex vigorously, then incubate the mixture at 65°C for 10 minutes.**
   Do not mix PX1 Buffer and RNase A prior to use. Invert 2-3 times during 65°C incubation.

3. **Add 1.3 ml of PX2 Buffer to the lysate, vortex, and incubate on ice for 5 minutes.**

4. **Apply lysate to the Shearing tube sitting in a Collection tube and centrifuge at full speed (about 3000 rpm or 2500 x g) for 2 minutes. Transfer flow-through sample from the Collection tube to a new tube (not provided).**
   Avoid pipetting any debris or pellet in the collection tube.

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5. **Add 0.5 volume of PX3 Buffer and 1 volume of 96-100% ethanol to the clear lysate and mix by pipetting.**
   For example: If 4.5 ml clear lysate collected, add 2.25 ml PX3 Buffer and 4.5 ml ethanol.

6. **Apply 6.5 ml of the ethanol added sample (including any precipitate) from step 5 to a Plant Genomic DNA Maxi Column sitting in a Collection tube, close the cap, centrifuge at full speed for 3 minutes, and discard the filtrate.**
   If the solution remains above the membrane, centrifuge again.

7. **Repeat step 6 for rest of the sample.**

8. **Wash the column twice with 5 ml of WS Buffer by centrifuging at full speed for 3 minutes and discard the filtrate.**
   Add 200 ml of ethanol (96-100%) to the WS Buffer bottle when first open the bottle.

9. **Centrifuge at full speed for 5 minutes to remove traces of WS Buffer.**

10. **Transfer the column to a new 15 ml tube (not provided), add 2 ml of 65°C TE or ddH_2O, and centrifuge at full speed for 5 minutes to elute DNA.**

11. **Store DNA at -20°C.**

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