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SUPPLEMENTARY PROTOCOL

Ver 1.0

KIT-9230

PrimeMag Plant Genomic DNA Extraction Kit

A support protocol is an extra guide provided with the main protocol to help users use the product with special or less common sample types, workflows, or conditions. It helps expand the use of the main kit by giving tested methods for other applications, while still ensuring good performance and quality. It doesn't replace the main protocol but offers extra help for different situations.

List of Supplementary Protocol for KIT-9230

1. Mushroom

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Materials Supplied by Users

- ✓ Centrifuge at speed of 10,200 rpm – 14,800 rpm
- ✓ Thermomixer with temperature settings of 65 °C, 56 °C and room temperature (DLAB HM100-Pro Thermo Mix or equivalent)
- ✓ Magnetic Rack (1st BASE, KIT-MAG16A)
- ✓ Vortex mixer
- ✓ 2 mL microcentrifuge tubes
- ✓ 1.5 mL microcentrifuge tubes
- ✓ Isopropanol (IPA)
- ✓ Absolute ethanol (≥ 99.5%)

Protocol

Preparation

I. Prepare 50 mg (up to 150 mg) of pulverized plant tissue using liquid nitrogen.

Tips:

- ✓ *To obtain the best result from plant nucleic acid extraction, bring liquid nitrogen to plant field to snap-freeze the plant tissue immediately during sample collection before process in laboratory.*
- ✓ *Pulverize plant tissue in liquid nitrogen using a set of sterilized mortar and pestle.*
- ✓ *Replenish the liquid nitrogen in the mortar 2 to 3 times and continue to grind sample until a fine, homogenous powder is obtained.*

II. Set thermomixer to 65 °C.

III. Freshly prepare 80% ethanol.
Example: For 1 prep, to prepare 600 µL 80% ethanol, add 480 µL into 120 µL of dH_2O

IV. Add absolute ethanol (≥ 99.5%) to WB1 Buffer as following:

Kit Preps	WB1 Buffer	Ethanol to be added
10 preps	4 mL	6 mL
48 preps	16 mL	24 mL
96 preps	30 mL	45 mL

Lysis

1. Transfer 50 mg of pulverized plant sample into 2 mL tube. Add **700 µl Plant Lysis Buffer**, **50 µl PG Buffer** and **10 µL RNase A into the sample tube.** Mix the sample by gently inverting the tube for 1 minute.
   Note: *Always add Plant Lysis Buffer and PG Buffer separately and never premix the solution.*

2. Incubate the mixture at 65 °C for 30 minutes in Thermo Mix (e.g. DLAB HM100-Pro) while shaking at 1,000 rpm.

3. After the incubation, change the Thermo Mix to room temperature.

Binding

4. After 30 minutes of incubation from step 2, centrifuge the lysate at 16,160 x g at room temperature for 5 minutes.

5. Leave the tube (with centrifuged lysate) at room temperature for 10 - 15 minutes or until the top layer appears.

6. Vortex the **PlantMag Beads** for 10 seconds to ensure they remain in suspension before use.

7. Prepare the **binding mixture (BindMix)** in a 2 mL microcentrifuge tube as following:

Components	**BindMix Volume (per prep)**
Isopropanol (IPA)	360 µL
BD2 Buffer	120 µL
PlantMag Beads	30 µL

Note: *Mix isopropanol and Binding Buffer just before use. The freshly prepared mixture can be used within 1 hour.*

Binding	8. Transfer 480 µL of supernatant (middle layer) to the **BindMix** tube 9. Mix the mixture in the **BindMix** tube by pipetting up and down for 10 times and shake at 1,000 rpm in room temperature, for 5 minutes in Thermo Mix. 10. Place the tube on a magnetic rack (e.g. 1st BASE, KIT-MAG16A) for 2 minutes, or until the **PlantMag Beads** separation has been completed. 11. Remove and discard the supernatant.
Washing	12. Add **600 µL WB1 Buffer** (ethanol added) into the **PlantMag Beads** tube. 13. Shake at 1, 000 rpm in room temperature, for 5 minutes in Thermo Mix. 14. Place the tube on a magnetic rack (e.g. 1st BASE, KIT-MAG16A) for 1 minutes or until the **PlantMag Beads** separation has been completed. 15. Remove and discard the supernatant. 16. Add **600 µL 80% ethanol** into the **PlantMag Beads** tube. 17. Shake at 1, 000 rpm in room temperature for 5 minutes in Thermo Mix. 18. Place the tube on a magnetic rack for 1 minute or until the **PlantMag Beads** separation has been completed.

Washing	19. Remove and discard the supernatant. 20. Air-dry the **PlantMag Beads** at room temperature for 5 to 10 minutes. ***Note:*** *DO NOT over dry the beads. Over drying could result low DNA yield.*
Elution	21. Add **100 µL** of Elution Buffer into the **PlantMag Beads** tube. 22. Shake at 1,000 rpm at 56 ℃ for 5 minutes. 23. Place the tube on a magnetic rack for 1 minute or until the **PlantMag Beads** separation has been completed. 24. Transfer the supernatant with purified DNA to a new 1.5 mL tube. *25. Optional: If there is sediment forming in the DNA eluent, centrifuge at max. speed of 16,160 xg for 1 minute, transfer clear eluent into a new 1.5 mL tube. DNA concentration will be reduced after this step.*

Product Ordering Information

Product Number	Product Description	Remarks
KIT-9230-10	PrimeMag Plant Genomic DNA Kit	Sufficient for 10 preps.
KIT-9230-48	PrimeMag Plant Genomic DNA Kit	Sufficient for 48 preps.
KIT-9230-96	PrimeMag Plant Genomic DNA Kit	Sufficient for 96 preps.
KIT-MAG16A	1st BASE Magnetic Rack	For 16 tubes processing.