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PrimeWay Plant DNA Extraction Kit (KIT-9030-50)

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Molecular Biology Kits





PrimeWay Plant DNA Extraction Kit

Product No: KIT-9030-50

The PrimeWay Plant DNA Extraction Kit is a rapid and reliable kit to isolate high quality genomic DNA from plant samples. This kit offers 2 types of lysis buffer: **PLB1 Buffer** (CTAB Buffer) and **PLB2 Buffer** (SDS Buffer) to produce optimum processing with high yield and excellent quality of DNA. Furthermore, this kit is supplied with RNase A to remove RNA contamination presence during the isolation steps. It uses a silica-based spin column and is suitable to isolate and purify plant DNA within 30 minutes/prep with a high recovery rate. Thus, making it suitable for PCR, restriction analysis, enzymatic reactions, Southern blotting & etc.

For Research Use Only. Not for use in Diagnostic Procedures.

Kit Contents

No	Product	KIT-9030-50	Storage
1	PLB1 Buffer	25 mL	Room temperature (21 °C – 25 °C)
2	PLB2 Buffer	20 mL	
3	PPB3 Buffer	10 mL	
4	PPC Buffer	30 mL	
5	Wash Buffer P1	30 mL	
5	Wash Buffer P2	25 mL	
6	Elution Buffer	13 mL	
7	RNase A	6 mg	
8	PrimeWay Plant Filter (Violet)	50 pcs	
8	PrimeWay Plant Column (Green)	50 pcs	
9	Collection Tube	100 pcs	



Product Specification

	KIT-9030-50
Binding capacity	50 µg
Yield	1 – 30 µg
Sample size	≤ 100 mg wet weight / ≤ 20 mg dry weight
Elution	2 x 50 µL
Duration	~ 30 minutes

Materials Supplied by Users

- ✓ Ethanol (96-100%)
- ✓ Water bath or thermo block to set at 30 - 40°C and 65°C
- ✓ Autoclaved/ nuclease-free water to suspend RNase A
- ✓ Centrifuge, at speed of 11,000 ×g
- ✓ Vortex mixer
- ✓ 1.5 mL microcentrifuge tubes
- ✓ Pipette & pipette tips

Precautions for Users

- ✓ Highly recommended to read entire instruction manual prior to start the plant DNA extraction especially (if) this kit is used for the first time.
- ✓ Some buffer of this kit contains irritants. Handle with care and avoid contact with skin. In case of contact, wash skin with a copious amount of water; seek medical attention.
- ✓ Always wear a lab coat, disposable gloves, and surgical mask.

Plant Sample Storage Method

- ✓ Plant sample can be stored in ethanol, frozen or lyophilize format after collection. Fresh plant sample can be stored overnight at 4 °C. If longer storage is needed, it is recommended to store at -20 °C.



Sample Homogenization Method

- ✓ Liquid nitrogen with mortar and pestle. Pre-cool mortar and pestle using liquid nitrogen. Grind the frozen sample to fine powder with liquid nitrogen. Keep the sample frozen throughout the grinding process.
- ✓ Steel beads. Place plant sample and 4 – 5 beads (7 mm) into a 15 mL tube. Freeze the tube in liquid nitrogen for 10 seconds and vortex for 30 seconds. Repeat the step until fine powder is obtained. DO NOT add liquid nitrogen into the tube.

Lysis Buffer Tested on Plant

Plant species	Part	PLB1	PLB2
<i>Abies alba</i> (fir)	Needle	/	/
<i>Acer griseum</i>	Leaf	/	/
<i>Allium porrum</i> (leek)	Leaf	/	/
<i>Amorphophallus titanum</i>	Leaf	/	Not tested
<i>Apium graveolens</i> (celery)	Corm	/	/
<i>Arabidopsis thaliana</i>	Leaf	/	/
<i>Artemisia vulgaris</i>	Leaf	/	/
<i>Artocarpus heterophyllus</i>	Leaf	/	/
<i>Azolla pinnata</i>	Leaf	/	Not tested
<i>Capsicum annuum</i>	Leaf	/	/
<i>Cleisostoma racemiferum</i>	Inflorescence rachis, silica-gel dried	/	Not tested
<i>Cocos nucifera</i> (coconut)	Leaf	/	/
<i>Doritis pulcherrima</i>	Leaf, silica-gel dried	/	Not tested
<i>Eichornia azurea</i>	Leaf	/	Not tested
<i>Elaeis guineensis</i> (oil palm)	Leaf	/	/
<i>Encephalartos natalensis</i>	Leaf	/	Not tested
<i>Euphorbia abyssinica</i>	Leaf	/	Not tested
<i>Euphorbia milii</i>	Leaf	/	Not tested
<i>Hordeum sp.</i> (barley)	Leaf	/	/
<i>Hydrilla verticillata</i>	Leaf	/	Not tested
<i>Lupinus sp.</i> (lupin)	Leaf	/	/
<i>Lycopersicon esculentum</i> (tomato)	Stem	/	/

Lists continue next page....



Plant species	Part	PLB1	PLB2
<i>Manihot esculenta</i> (cassava)	Leaf	/	/
<i>Ocimum basilicum</i>	Leaf	/	/
<i>Oryza sativa</i> (rice)	Leaf	/	/
<i>Pistia stratiotes</i>	Leaf	/	Not tested
<i>Pometia pinnata</i>	Leaf	/	Not tested
<i>Pteridium sp.</i>	Leaf	/	Not tested
<i>Pterocarya fraxinifolia</i>	Leaf	/	Not tested
<i>Rosa sp.</i> (rose)	Leaf	/	/
<i>Secale sp.</i> (rye)	Leaf	/	/
<i>Solanum lycopersicum</i> (tomato)	Leaf	/	/
<i>Spirodela polyrhiza</i>	Leaf	/	Not tested
<i>Stereochilus sp.</i>	Leaf, silica-gel dried	/	Not tested
<i>Trachycarpus takil</i>	Leaf	/	Not tested
<i>Trichoglottis sp.</i>	Leaf, silica-gel dried	/	Not tested
<i>Triticum aestivum</i> L (wheat)	Leaf	/	/
<i>Vigna radiata</i> (mung bean)	Root	/	/
<i>Zea mays</i> (maize)	Leaf	/	/
<i>Zea mays</i> (maize)	Grain, dried, ground coarsely	/	/

Algae species	Part	PLB1	PLB2
<i>Caulerpa lentillifera</i>	Frond	/	Not tested
<i>Caulerpa microphysa</i>	Frond	/	Not tested
<i>Caulerpa taxifolia</i>	Frond	/	Not tested
<i>Gracilaria sp.</i>	Whole algae	/	Not tested
<i>Ulva sp.</i>	Whole algae	/	Not tested

Other species	Part	PLB1	PLB2
<i>Cordyceps militaris</i>	Fruiting body	/	/
<i>Ganoderma lucidum</i>	Fruiting body (dried)	/	/
<i>Hypsizygus tessellatus</i> (Bunapi-shimeji)	Fruiting body	/	/
<i>Lentinula edodes</i>	Fruiting body (dried)	/	/



Protocol

Preparation	<ol style="list-style-type: none"> i. Add 600 µL of autoclaved/ nuclease-free water (not provided) to RNase A and vortex. Make multiple aliquots to prevent frequent freeze-thaw. Short spin to bring down the liquid and store it at 4 °C for ≤ 3 months or -20 °C for ≤ 1 year. ii. Add 100 mL of ethanol (96 – 100%) into Wash Buffer P2. Wash Buffer P2 is stable at room temperature (18 – 25 °C). iii. Lysis PLB1 Buffer & PLB2 Buffer may have precipitation. If precipitation occurs, incubate the bottle at 30 - 40 °C for few minutes or until the precipitate dissolved completely. iv. Pre-heat the Elution Buffer at 65 °C before use.
Sample	<ol style="list-style-type: none"> 1. Homogenize up to 100 mg wet weight / 20 mg dry weight plant material to fine powder. <i>Note: Refer page 3 for Sample Homogenization Method.</i> 2. Transfer the fine powder to a new 1.5 mL microcentrifuge tube*. <i>*Proceed with either 2A Lysis (PLB1 Buffer) OR 2B Lysis (PLB2 Buffer)</i>
Lysis (CTAB)	<p>2A Lysis (PLB1 Buffer)</p> <ol style="list-style-type: none"> i) Add 400 µL PLB1 Buffer and resuspend it completely by vortexing. <i>Note: If sample too dry and cannot be resuspended, add additional PLB1 Buffer. Increase the volume of RNase A (Step 2a ii) and PPC Buffer (Step 7) proportionately.</i> ii) Add 10 µL RNase A solution and mix thoroughly. iii) Incubate the mixture at 65 °C for 10 minutes. <i>Note: This incubation time can be increased to 30 – 60 min based on type of plant.</i> <p>Proceed to Step 3.</p>
<div style="border: 1px solid black; padding: 5px; display: inline-block; color: red; font-weight: bold;">OR</div>	



Lysis (SDS)	<p>2B Lysis (PLB2 Buffer)</p> <ol style="list-style-type: none"> i) Add 300 μL PLB2 Buffer and resuspend it completely by vortexing. <i>Note: If sample too dry and cannot be resuspended, add additional PLB2 Buffer. Increase the volume of RNase A (Step 2B ii), PPB3 Buffer (Step 2B iv) and PPC Buffer (Step 7) proportionately.</i> ii) Add 10 μL RNase A solution and mix thoroughly. iii) Incubate the mixture at 65 °C for 10 minutes. <i>Note: This incubation time can be increased to 30 – 60 min based on type of plant.</i> iv) Add 75 μL PPB3 Buffer and mix thoroughly. Incubate on ice for 5 minutes for precipitation. <p>Proceed to Step 3.</p>
Filtration	<ol style="list-style-type: none"> 3. Centrifuge the crude lysate at 11,000 x g for 5 minutes. 4. Place the PrimeWay Plant Filter (Violet) into a new Collection tube. 5. Transfer the supernatant into the PrimeWay Plant Filter (Violet) and centrifuge at 11,000 x g for 2 minutes. <i>Note: Repeat the centrifugation at maximum speed if there are remaining supernatant in the column.</i> 6. Transfer the flow-through into a new 1.5 ml microcentrifuge tube. Discard the PrimeWay Plant Filter (Violet). <i>Note: Avoid transferring any visible pellet, only transfer clear supernatant.</i>
Binding	<ol style="list-style-type: none"> 7. Add 450 μL PPC Buffer into the tube. Mix thoroughly by vortexing or pipetting up and down. 8. Place the PrimeWay Plant Column (Green) into a new Collection tube. Transfer maximum 700 μL of lysate into the PrimeWay Plant Column. 9. Centrifuge at 11,000 x g for 1 minute and discard the flow-through. Place the column back to the Collection tube. Repeat centrifugation if there is remaining volume in the column.



Washing	<ol style="list-style-type: none">10. Add 400 μL Wash Buffer P1 into the column. Centrifuge at $11,000 \times g$ for 1 minute and discard the flow-through.11. Add 700 μL Wash Buffer P2 into the column. Centrifuge at $11,000 \times g$ for 1 minute and discard the flow-through.12. Add 200 μL Wash Buffer P2 into the column. Centrifuge at $11,000 \times g$ for 2 minute and discard the flow-through.
Elution	<ol style="list-style-type: none">13. Place the PrimeWay Plant Column into a new 1.5 mL microcentrifuge tube.14. Add 50 μL pre-heated Elution Buffer (65 °C) to the center of the column membrane. Let the column stand at room temperature for 5 minutes.15. Centrifuge the tube at $11,000 \times g$ for 1 minute to elute the DNA.16. Repeat Step 13 & 14 for second elution. Total elution volume is 100 μL. Store the purified DNA in -20 °C.



Troubleshooting Guidelines

Problems	Possible Reason	Recommended Action
Low DNA yield	Poor homogenization of sample	Homogenize using steel beads or by using liquid nitrogen (with pestle & mortar) until fine powder is obtained.
	Choice of lysis buffer	Performance of PLB1 buffer and PLB2 buffer varies from plant species. Compare both lysis buffers for best lysis efficiency to your plant sample.
	Low volume of lysis buffer	Increase the lysis buffer volume if dry sample absorb too much buffer. Also, increase the reagent volume mentioned in Protocol 2A or 2B proportionately.
	Insufficient lysis	Increase incubation time in lysis buffer (up to overnight)
	DNA Elution	Ensure that the Elution is preheated and is added to the center of the column.
PrimeWay Plant Filter or PrimeWay Plant Column is clogged	Too much of sample material	Follow the recommended sample weight.
		Increase centrifugation time.
DNA is degraded.	Sample/ Component(s) of the kit is contaminated with DNase	Prepare new sample and extract fresh DNA.
		Purchase a new kit.
DNA quality is low	Salt or ethanol carry over	Centrifuge with a maximum speed of 11,000 xg. Higher speed may shear the DNA
		Ensure Washing Step is performed accordingly, and membrane is thoroughly dried before adding the elution buffer.

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