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PrimeWay Plant DNA Extraction Kit (KIT-9030)

PrimeWay Plant DNA Extraction Kit

Product No: KIT-9030

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-9030-10 10 preps	KIT-9030-50 50 preps	KIT-9030-250 250 preps
1	PLB1 Buffer	5 mL	25 mL	125 mL
2	PLB2 Buffer	4 mL	20 mL	100 mL
3	PPB3 Buffer	1 mL	10 mL	25 mL
4	PPC Buffer	5 mL	30 mL	125 mL
5	Wash Buffer P1	5 mL	30 mL	125 mL
6	Wash Buffer P2	2 mL	25 mL	50 mL
7	Elution Buffer	1.2 mL	13 mL	30 mL
8	RNase A	1.5 mg	6 mg	2 x 15 mg
9	PrimeWay Plant Filter	10 pcs	50 pcs	5 x 50 pcs
10	PrimeWay Plant Column	10 pcs	50 pcs	5 x 50 pcs
11	Collection Tube	2 x 10 pcs	100 pcs	10 x 50 pcs

Storage

This kit should be stored at room temperature (21 – 25 °C). After reconstitution, store RNase A solution at 4 °C or - 20 °C (for longer storage).

Product Specification

	KIT-9030
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.

Before Start

✓ Add **autoclaved/ nuclease-free water** (not provided) to **RNase A** as follow:

P/No	Nuclease-free water to be added
KIT-9030-10	150 µL
KIT-9030-50	600 µL
KIT-9030-250	1.5 mL per tube

Vortex to mix. 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.

✓ Add **ethanol (96 – 100%)** into **Wash Buffer P2** as follow**:**

P/No	Ethanol to be added
KIT-9030-10	8 mL
KIT-9030-50	100 mL
KIT-9030-250	200 mL

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

Lists continue next page….

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

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

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

Protocol

Preparation

- ✓ 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.
- ✓ Pre-heat the **Elution Buffer** at 65 °C before use.

Sample

1. Homogenize up to **100 mg wet weight / 20 mg dry weight** plant material to fine powder.
   Note: *Refer page 3 for Sample Homogenization Method.*
2. Transfer the fine powder to a new 1.5 mL microcentrifuge tube*.
   Proceed with **either **2A Lysis (PLB1 Buffer)** **OR** **2B Lysis (PLB2 Buffer)***

Lysis (CTAB)

2A Lysis (PLB1 Buffer)

i) Add **400 µL PLB1 Buffer** and resuspend it completely by vortexing.
   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.*

ii) Add **10 µL RNase A solution** and mix thoroughly.

iii) Incubate the mixture at 65 °C for 10 minutes.
   Note: *This incubation time can be increased to 30 – 60 min based on type of plant.*

Proceed to Step 3.

OR

Lysis (SDS)

2B Lysis (PLB2 Buffer)

i) Add **300 µL PLB2 Buffer** and resuspend it completely by vortexing.
***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.*

ii) Add **10 µL RNase A solution** and mix thoroughly.

iii) Incubate the mixture at 65 °C for 10 minutes.
***Note**: This incubation time can be increased to 30 – 60 min based on type of plant.*

iv) Add **75 µL PPB3 Buffer** and mix thoroughly. Incubate on ice for 5 minutes for precipitation.

Proceed to Step 3.

Filtration

3. Centrifuge the crude lysate at 11,000 x *g* for 5 minutes.

4. Place the **PrimeWay Plant Filter** into a new Collection tube.

5. Transfer the supernatant into the **PrimeWay Plant Filter** and centrifuge at 11,000 × *g* for 2 minutes.
***Note**: Repeat the centrifugation at maximum speed if there are remaining supernatant in the column.*

6. Transfer the flow-through into a new 1.5 mL microcentrifuge tube. Discard the PrimeWay Plant Filter.
***Note**:* Avoid transferring any visible pellet, only transfer clear supernatant.

Binding

7. Add **450 µL PPC Buffer** into the tube. Mix thoroughly by vortexing or pipetting up and down.

8. Place the **PrimeWay Plant Column** into a new Collection tube. Transfer maximum 700 µL of lysate into the PrimeWay Plant Column.

9. Centrifuge at 11,000 × *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	10. Add **400 µL Wash Buffer P1** into the column. Centrifuge at 11,000 × *g* for 1 minute and discard the flow-through. 11. Add **700 µL Wash Buffer P2** into the column. Centrifuge at 11,000 × *g* for 1 minute and discard the flow-through. 12. Add **200 µL Wash Buffer P2** into the column. Centrifuge at 11,000 × *g* for 2 minutes and discard the flow-through.
Elution	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 × *g* for 1 minute to elute the DNA. 16. Repeat Step 13 - 15 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 buffer 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.
	Too much precipitate	Centrifuge to bring down the precipitation. Transfer only the cleared lysate to PrimeWay Plant Filter.
DNA is degraded.	Sample/ Component(s) of the kit is contaminated with DNase	Prepare new sample and extract fresh DNA.
		Purchase a new kit.
	Centrifugation speed is too high	Centrifuge with a maximum speed of 11,000 × *g*. Higher speed may shear the DNA.
DNA quality is low	Salt or ethanol carry over	Ensure Washing Step is performed accordingly, and membrane is thoroughly dried before adding the elution buffer.

Please contact us at https://base-asia.com/contact/ for more information.