1st BASE
YOUR RESEARCH, OUR BUSINESS

Ver. 2.0

SEPa Plant DNA Isolation Reagent Kit (KIT-9101-125ml)

SEPa Plant DNA Isolation Reagent Kit

Product No: KIT-9101-125ml

This product allows high quality DNA extraction from plant tissues, especially those rich in polyphenolics or polysaccharides. It is an organic solution-based extraction method which utilizes cationic detergent to extract nucleic acid for downstream applications that require high quality inputs of genomic DNA. This product includes RNA removal components to produce genomic DNA that is free from RNA contamination. It is an ideal kit to perform high quality genomic DNA extraction from various plant tissues before PCR, Restriction Enzyme Digestion, Southern Blots, Sanger Sequencing and Next-Generation Sequencing (NGS).

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

Kit Contents

No	Product	KIT-9101-125ml	Storage
1	Plant Lysis Buffer	125 mL	Room temperature (21 – 25°C)
2	10 mM Tris Buffer, pH 8.5	15 mL	
3	Diluent Solution	50 mL	
4	3 M Sodium Acetate, pH5.2	1.5 mL	
5	Binding Enhancer	3 x 1 mL	-20°C
6	RNase A Solution (10 mg/mL)	700 µL	

Storage

This kit will be delivered at room temperature (21 – 25 °C) and dry ice. Upon receipt, store the kit components according to the storage temperatures indicated on the box label.

Product Specification

	KIT-9101-125ml
No. of reactions	Up to 120 preps
Sample size	200 mg pulverized plant tissues
Elution	100 – 200 µL
Duration	2 ½ to 3 hours

Materials Supplied by Users

- ✓ Water bath or thermo block
- ✓ Refrigerated centrifuge at speed of 10,200 rpm – 14,800 rpm
- ✓ Rotary Mixer (e.g. Elmi Intelli-Mixer RM-2M at mode F99, RPM 45)
- ✓ 2 mL microcentrifuge tubes (4x for each sample)
- ✓ Chloroform:isoamyl alcohol 24:1
- ✓ Pre-cold isopropanol
- ✓ Pre-cold fresh 70% ethanol
- ✓ Beta-mercaptoethanol
- ✓ Pre-cold absolute ethanol (optional for additional RNase treatment)
- ✓ Room temperature 70% ethanol (optional for additional RNase treatment)

Precautions for Users

- ✓ Plant lysis buffer 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.

Protocol

Preparation

I. Prepare 200 mg of pulverized plant tissue by 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 water bath or thermo block to 65 °C. Pre-warm Plant Lysis Buffer at 65 °C before use.

III. Set the refrigerated centrifuge to 4 °C before use.

IV. Prepare fresh ice-cold 70% ethanol.

V. Prepare ice-cold isopropanol.

Lysis

1. Transfer 200 mg of pulverized plant sample into 2 mL tube. Add **1 mL of 65°C pre-warmed Plant Lysis Buffer**, **25 µL Binding Enhancer** and **20 µL Beta-mercaptoethanol** (not provided) into the plant sample. Invert and vortex briefly to mix.

2. Incubate the mixture for 30 minutes at 65 °C in water bath/ thermo block. Invert tube to mix every 5 – 10 minutes.

Washing

3. Centrifuge at 14,800 rpm for 10 minutes at 4 °C to remove non-soluble debris. Transfer the supernatant (~ 900 µL) into a new 2 mL tube.

4. Add equal volume of **chloroform:isoamyl alcohol 24:1** (~ 900 µL).

5. Invert the mixture 20 times and centrifuge at 13,000 rpm for 15 minutes at 4 °C. Transfer supernatant (~ 600 µL) to a new 2 mL tube.

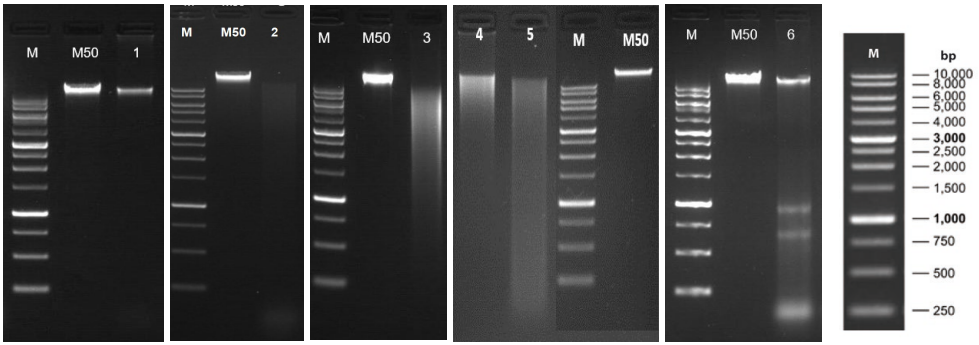
6. Add **300 µL Diluent Solution** into the supernatant. Invert the mixture 20 times to mix gently.

RNA Removal	7. Add **5 µL RNase A Solution** (10 mg/mL). Invert the mixture and spin down. Incubate at 37 °C for 30 minutes. 8. Add **equal volume of chloroform:isoamyl alcohol 24:1** (~ 900 µL). 9. Invert the mixture 20 times. Then shake at room temperature for 20 minutes on Rotary Mixer (e.g. Elmi intelli-Mixer RM-2M at the speed mode of F99, RPM 45). *Note: If 2 layers still observed during mixing, increase the mixing speed until there is no separation.* 10. Centrifuge at 13,000 rpm for 15 minutes at 4 °C. Transfer supernatant (~ 750 µL) into a new 2 mL tube.
Purification	11. Add **900 µL ice cold isopropanol** (not provided), invert to mix and incubate at -20 °C for 10 – 20 minutes. 12. Centrifuge at 13,000 rpm for 10 minutes at 4 °C. Discard supernatant. *Tips: The nucleic acid pellet is formed at this stage.* 13. Add **1 mL ice-cold 70% ethanol** (not provided) to the pellet. Dislodge the pellet by flicking with a finger gently. 14. Centrifuge at 10,200 rpm for 5 minutes at 4 °C. Discard supernatant. 15. Invert tube onto a paper towel to air dry the pellet for 3 min.
Elution	16. Re-suspend the dried DNA pellet in 1**00 – 200 µL of 10 mM Tris buffer (pH8.5)** or **Diluent Solution**. 17. Measure the Optical Density (OD) reading using spectrophotometer. Dilute the nucleic acid to 25 – 50 ng/µL. Use 2 µL of this diluted nucleic acid to check for its DNA integrity using any setup of electrophoresis. Optional: Should RNA contamination be observed, proceed to the RNase treatment. The additional RNase treatment will reduce the total yield of the purified DNA.

Protocol – Additional RNase Treatment (Optional)

Repeat RNase Treatment	1. For each 100 µL of DNA, add **1 µL RNase A Solution (10 mg/mL)**. *Tips:* *Top-up the DNA using the provided Diluent Solution until 100* µL *before each additional RNase treatment.* 2. Incubate the reaction at 37 °C for 30 minutes.
Purification	3. Add **1/10 volume (~ 10 uL) of 3M Sodium Acetate (pH 5.2)**. Invert the mixture 20 times to mix gently and spin down. 4. Add **2x volumes (~ 220 uL) of ice-cold absolute ethanol** (not provided). Incubate overnight in a -20 °C freezer. 5. Centrifuge at 14,000 rpm for 15 minutes at 4 °C. Discard supernatant. 6. Add **1 mL room temperature 70% ethanol** (not provided). Invert the mixture 20 times to mix gently. 7. Centrifuge at 14,000 rpm for 5 minutes at 4 °C. Discard supernatant. 8. Invert tube onto a paper towel to air dry the pellet for 3 minutes.
Elution	9. Re-suspend the dried DNA pellet in **100 – 200 µL Diluent Solution**. 10. Measure the Optical Density (OD) reading using spectrophotometer. Dilute the nucleic acid to 25 – 50 ng/µL. Use 2 µL of this diluted nucleic acid to check for its DNA integrity using any setup of electrophoresis.

Agarose Gel Electrophoresis



2 µL gDNA was run on 1% TAE agarose gel at 100V for 60 min.

Label	Description
M	1st BASE ExactMark 1kb DNA Ladder, Ready-To-Use (P/N: BIO-5140-50µg)
M50	Positive control 50 ng
1	Good quality of gDNA, suitable for all application including NGS
2	gDNA with low yield
3, 4 & 5	gDNA with different degree of degradation
6	gDNA contaminated with RNA

The kit has been tested with wide range of plant samples in the table below:

Ananas comosus (pineapple flesh)	*Callicarpa americana* (beautyberry)	*Ficus elastica* (rubber tree leaf)	*Passiflora* (passion vines leaf/ fruit)
Baccaurea motleyana (rambai leaf)	*Carica papaya* (papaya leaf)	*Ganoderma lucidum* (Lingzhi mycelium/ fruiting body)	*Stevia rebaudiana* (candyleaf)
Brassica napus (rapeseed leaf)	*Elaeis guineensis* (oil palm leaf)	*Mangifera indica* (mango flesh)	

Troubleshooting Guidelines

Problems	Possible Reason	Recommended Action
Low yield of nucleic acid	Coarsely ground sample	Grind sample to a fine powder.
	The samples were incompletely homogenized or lysed.	Decrease the amount of starting material. Ensure the sample is completely immersed in the Plant Lysis Buffer to achieve total lysis. Vortex to suspend the sample (ensure that it is off the bottom of the tube) and reduce the size of clumps.
	The dried nucleic acid pellet was incompletely solubilized.	Increase the solubilization rate by gently pipetting the dried DNA pellet up and down with the provided 10 mM Tris buffer (pH8.5). You may also consider heating the Tris buffer to 55 °C to increase the solubility of DNA pellet.
Nucleic acid shows degradation	Samples were not immediately processed or frozen after collection.	Sample must be processed or frozen immediately after collection.
	Sample preparations were stored at the incorrect temperature.	Store samples at -60 °C to -80 °C after collection and for long-term storage.
The DNA is contaminated	The interphase/organic phase is pipetted up with the aqueous phase during washing/RNA removal step	Do not attempt to transfer the entire aqueous layer after phase separation during washing/RNA removal step.
	Excess RNA remains after the sufficient RNase treatment	For some plant samples, Beta-mercaptoethanol may interact with certain metabolites which can cause the failure in the extraction of DNA. Repeat the extraction and do not add any Beta-mercaptoethanol.
The DNA A260/280 is low	Sample was homogenized in an insufficient volume of Plant Lysis Buffer	Add the appropriate amount of Plant Lysis Buffer for your sample type
	The interphase/organic phase is pipetted up with the aqueous phase during washing/RNA removal step	Do not attempt to transfer the entire aqueous layer after phase separation during washing/RNA removal step.

Product Ordering Information

Product Number	Product Description	Remarks
KIT-9101-125ml	SEPa Plant DNA isolation Reagent Kit	Sufficient for 125 preps.
K.BUF-9102-125ml	Plant Lysis Buffer	Sufficient for 125 preps.
K.RGT-9101-1ml	Binding Enhancer for Plant Lysis, 1mL	Sufficient for 40 preps.
K.RGT-9105-50ml	Diluent Solution	Sufficient for 100 preps.
K.RGT-9106-1.5ml	3M Sodium Acetate, pH5.2	Sufficient for 150 preps.
K.RGT-9110-1ml	RNase A Solution, 100 mg/mL, 1mL	Sufficient for up to 200 preps.
MBS-6102	RNA LabChip Analysis: Eukaryotic (Plant), 18S and 25S. Price per chip (up to 12 samples)	
MBS-6104	RNA Preparation: Reverse Transcription PCR	
MBS-6106	RNA Preparation: in-vitro transcription	

Customization of nucleic acid extraction buffer for your choice of organism is available. Please contact us at https://base-asia.com/contact/ for more information.