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Ver. 2.0

SEPa Plant RNA Isolation Reagent Kit (KIT-9102-125ml)

SEPa Plant RNA Isolation Reagent Kit

Product No: KIT-9102-125ml

This product allows high quality RNA 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 total RNA. The product includes DNA removal components to produce total RNA that free from DNA contamination. It is an ideal kit to perform high quality total RNA extraction from various plant tissues before qPCR, Northern Blot analysis, poly(A)+ selection, in vitro transcription/ translation, and Next-Generation Sequencing (NGS).

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

Kit Contents

No	Product	KIT-9102-125ml	Storage
1	Plant Lysis Buffer	125 mL	Room temperature (21 – 25 °C)
2	8 M Lithium Chloride	20 mL	
3	Nuclease-free water	15 mL	
4	Binding Enhancer	3 x 1 mL	-20°C
5	DNase I (1U/µL)	250 µL	
6	DNase Buffer with $MgCl_2$ (10X)	1.25 mL	
7	DNase Removal Solution	500 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-9102-125ml
No. of reactions	Up to 40 preps
Sample size	200 mg pulverized plant tissues
Elution	50 – 100 µL
Duration a) RNA Extraction b) DNase Treatment	 a) ~2 hours with overnight incubation b) 35 minutes

Materials Supplied by Users

- ✓ Water bath or thermo block
- ✓ Refrigerated centrifuge, at speed of 10,200 – 14,800 rpm
- ✓ 15 mL centrifuge tube (1x for each sample)
- ✓ 2.0 mL micro-centrifuge tubes (10x for each sample)
- ✓ 1.5 mL micro-centrifuge tubes (optional for DNase treatment: 2x for each sample)
- ✓ Chloroform:isoamyl alcohol 24:1
- ✓ Pre-cold fresh 70% ethanol
- ✓ Beta-mercaptoethanol

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 the 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.
Lysis	1. Transfer 200 mg of pulverized plant sample into 15 mL tube. Add **3 mL** of **65 °C pre-warmed Plant Lysis Buffer**, **75 µL Binding Enhancer** and **60 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. 3. By using a wide bore pipette tip, aliquot 1 mL sample into each 2 mL tube. Total of 3 x 2 mL tubes are required for each sample in this step.
Washing	4. 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. 5. To each 2 mL tube, add equal volume of **chloroform:isoamyl alcohol 24:1** (~ 900 µL). 6. 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. 7. To each 2 mL tube, add equal volume of **chloroform:isoamyl alcohol 24:1** (~ 600 µL).

Washing	8. Invert the mixture 20 times and centrifuge at 13,000 rpm for 15 minutes at 4 °C. Pool the supernatant (~450 µL) from 3 tubes of the same samples into a new 2 mL tube.
Precipitation	9. Add **1/3 volume of 8 M Lithium Chloride** (~ 450 µL) to the pooled supernatant. Invert gently to mix. 10. Incubate the mixture overnight at 4 °C. 11. Centrifuge at 13,000 rpm for 15 minutes at 4 °C. Discard supernatant. *Tips: The nucleic acid pellet is formed at this stage.* 12. Add **1 mL ice-cold 70% ethanol** (not provided) to the pellet. Dislodge the pellet by flicking with a finger gently. 13. Centrifuge at 10,200 rpm for 5 minutes. Discard supernatant. 14. Invert tube onto a paper towel to air dry the pellet for 3 minutes.
Elution	15. Re-suspend the dried RNA pellet in **50 – 100 µL nuclease-free water**. 16. 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 RNA integrity using any setup of electrophoresis and / or RNA Labchip. Optional: Shall DNA contamination be observed, proceed to the DNase treatment.

Protocol – DNase Treatment (Optional)

DNase Treatment

1. According to different amount of total RNA, prepare the DNase treatment reaction according to **Table 1** in nuclease-free 1.5 mL tubes.

Table 1: DNase Treatment for different amount of total RNA.

Component	Amount A	Amount B	Amount C
RNA sample	Up to 8.5 µL (5 pg – 2 µg)	Up to 42.5 µL (25 pg – 10 µg)	Up to 85 µL (50 pg – 20 µg)
10X DNase Buffer with $MgCl_2$	1 µL	5 µL	10 µL
DNase I (1 U/µL)	0.5 µL	2.5 µL	5 µL
Nuclease-free water	Adjust to reach the final volume	Adjust to reach the final volume	Adjust to reach the final volume
Total Final Volume	10 µL	50 µL	100 µL

2. Incubate the reaction at 37 °C for 30 minutes.

DNase Removal

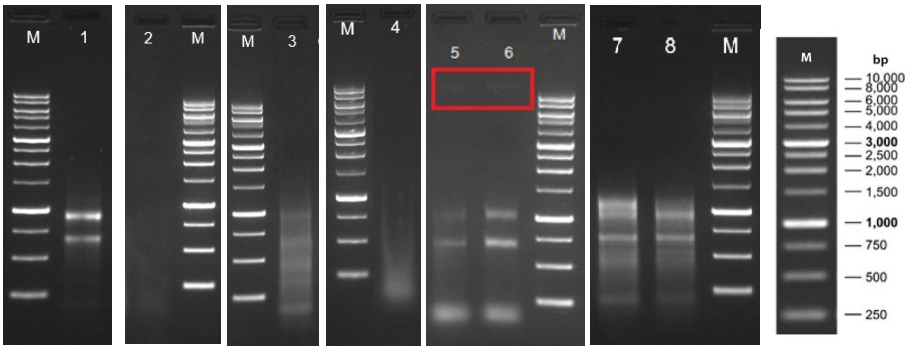
3. For each µL of **DNase I** used in the DNase treatment reaction, add **2 µL DNase Removal Solution**.
   Tips: Prior using the DNase Removal Solution, vortex the solution until it is completely suspended.

4. Incubate the reaction at room temperature for 2 minutes. Invert 2 to 3 times gently to mix.

5. Centrifuge at 3,500 rpm for 1 minute to pellet the DNase Removal Solution.

6. Transfer the supernatant (which is DNase-free total RNA) into a new nuclease-free 1.5 mL tube.
   Tips: Do not disturb the pelleted DNase Removal Solution.

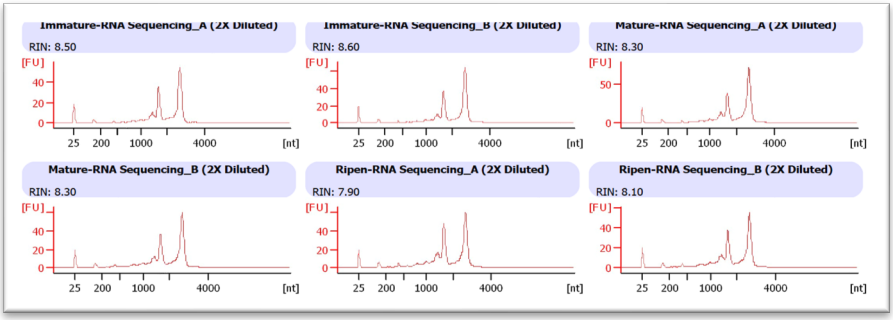
Agarose Gel Electrophoresis



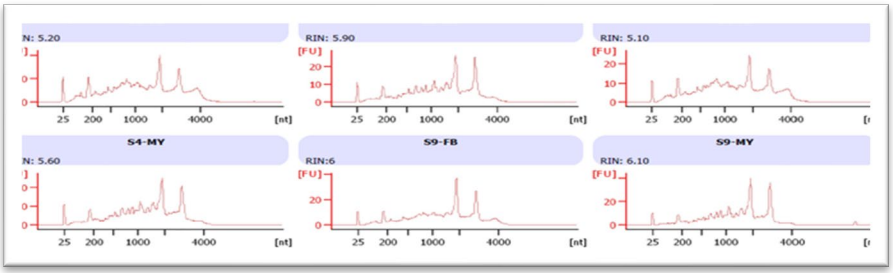
2 µL of total RNA were run on 1% TAE agarose gel at 100V for 60 minutes.

Label	Description
M	1st BASE ExactMark 1kb DNA Ladder, Ready-To-Use (P/N: BIO-5140-50µg)
1	Good quality of total RNA, suitable for all application including NGS
2	Total RNA with low yield
3 & 4	Total RNA with different degree of degradation
5 & 6	Total RNA with contamination of gDNA
7 & 8	Good quality of total RNA from difficult samples (slimy plant tissues)

RNA Labchip Analysis



LabChip Analysis: Eukaryotic (Plant), 18S and 25S for RNA integrity confirmation from regular plant RNA isolation. Good quality of total RNA, suitable for all application including NGS.



LabChip Analysis: Eukaryotic (Plant), 18S and 25S for RNA integrity confirmation from difficult plant RNA isolation. Best possible quality of total RNA, suitable for all application including NGS.

The kit has been tested with wide range of plant samples:

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)	*Theobroma cacao* (cocoa pulp and placenta)

Troubleshooting Guidelines

Problems	Possible Reason	Recommended Action
Low yield of nucleic acid	Coarsely ground sample	Grind sample to a fine powder.
	The samples were not completely 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 not completely solubilized.	Increase the solubilization rate by gently pipetting the dried RNA pellet up and down with the provided nuclease-free water.
Nucleic acid shows degradation	Samples were not immediately processed or frozen after collection.	Sample must be processed or frozen immediately after collection. Store sample at -80 °C after collection and for long-term storage.
	Sample allowed to thaw before extracting with Plant Lysis Buffer	Keep sample at -80 °C until Plant Lysis Buffer is added and the powder is dispersed in the buffer.
	Sample preparations were stored at the incorrect temperature	Store RNA samples at -60 °C to -80 °C.
The RNA is contaminated	The interphase/organic phase is pipetted up with the aqueous phase during washing step	Do not attempt to draw off the entire aqueous layer after phase separation during washing step.
The RNA 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 step	Do not attempt to draw off the entire aqueous layer after phase separation during washing step.

Product Ordering Information

Product Number	Product Description	Remarks
KIT-9102-125ml	SEPa Plant RNA isolation Reagent Kit	Sufficient for 41 preps.
K.BUF-9102-125ml	Plant Lysis Buffer	Sufficient for 41 preps.
K.RGT-9101-1ml	Binding Enhancer for Plant Lysis, 1mL	Sufficient for 40 preps.
K.RGT-9102-10ml	Lithium Chloride Solution, 8 M	Sufficient for 22 preps.
K.RGT-9103-250U	DNA Removal Reagents	Sufficient for 50 – 500 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.