SHANKARANARAYANA LIFE SCIENCES
A NEW GENERATION COMAPANY

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Snls
A NEW GENERATION COMPANY

SN Plant/Feed DNA extraction Kit

Column Based

Innovation Speaks

SN PLANT/FEED DNA EXTRACTION KIT

(Column Based)

DESCRIPTION:

SN PLANT/FEED DNA Extraction Kit is a specialized tool used to isolate high purity DNA from various PLANT/FEED samples. It is a column based method which is reliable and efficient way to isolate high quality of DNA from PLANT/FEED samples. Kit is essential for various molecular biology and genetic research applications.

CONTENTS OF KIT:

Sl. No	Components	Volume		
		5 Rxn	50 Rxn	100 Rxn
1	RS Buffer	2.5ml	25ml	50ml
2	LS Buffer	3ml	30ml	60ml
3	STB Buffer	2.5ml	25ml	50ml
4	W1 Buffer	1.5ml	15ml	30ml
5	Elution Buffer	250µl	2.5ml	5ml
6	SN Spin Columns	5 No.	50 No.	100 No.

NOTE: Preparation for first use after receiving the kit (Add 100% Ethanol to Wash Buffer) (5Rxn: 1ml, 50Rxn:10 ml, and 100Rxn: 20ml) mix well and store the buffer at room temperature.

- If Proteinase K shipped in lyophilized form, upon receiving resuspend with Nuclease free water (5Rxn:100µl,50Rxn: 1ml, 100Rxn: 2ml)
- If RNase A shipped in lyophilized form, upon receiving resuspend with Nuclease free water (5Rxn:50µl, 50Rxn:500µl, 100Rxn: 1ml)
- **Proteinase –K and RNase A, store at -20°C either liquid form or lyophilized form.**

REQUIRED MATERIALS NOT PROVIDED

- ✓ 100% Ethanol & 70 % Ethanol
- ✓ Dry bath
- ✓ 1.5ml Centrifuge tubes
- ✓ micro centrifuge
- ✓ Vortex

STORAGE / SHIPPING:

- Shipped at: Ambient Temperature.
- Storage: All Buffers can be stored at Room temperature.

SPECIFICATIONS:

Principle	Spin column
Recommended Input Amount: 200mg PLANT/FEED Sample	
Elution Volume : 50µl	
Purity: A260/280 - 1.8±0.1	
Compatible Down8treem Application: PCR, qPCR, Sequencing	
Expected Yield : depends upon the sample	

PROCEDURE:

1) Weigh 200mg of clean PLANT/FEED sample and transfer to mortar and pestle grind like a fine paste by adding 500µl of RS Buffer.
2) Transfer the above mixture to 1.5 ml centrifuge tube using a clean spatula.
3) Add 600 µl of LS buffer into the sample along with 20µl of Proteinase K and 10µl of RNase A Vortex for 1min.
4) Incubate at 60°C for 15mins with intermittent vertexing for 3-4 times to lysed the cells are completely. Centrifuge the tube for 2mins at 10,000rpm, then transfer the supernatant to a fresh centrifuge tube.
5) Add 500µl of 100 % ethanol to the supernatant, keep the tube for 2 mins at room temperature, and observe precipitation in the supernatant.
6) Transfer the precipitate to the Spin Column, then centrifuge at 15,000 rpm for 2mins and discard the flow-through in the collection tube.
7) Add 500µl of STB buffer to the SN spin column and centrifuge at 15,000 rpm for 1min, discard the flow through into the collection tube.
8) Add 500µL of W1 buffer solution to the SN spin column and centrifuge at 15,000 rpm for 1min, discard the flow through into the collection tube.

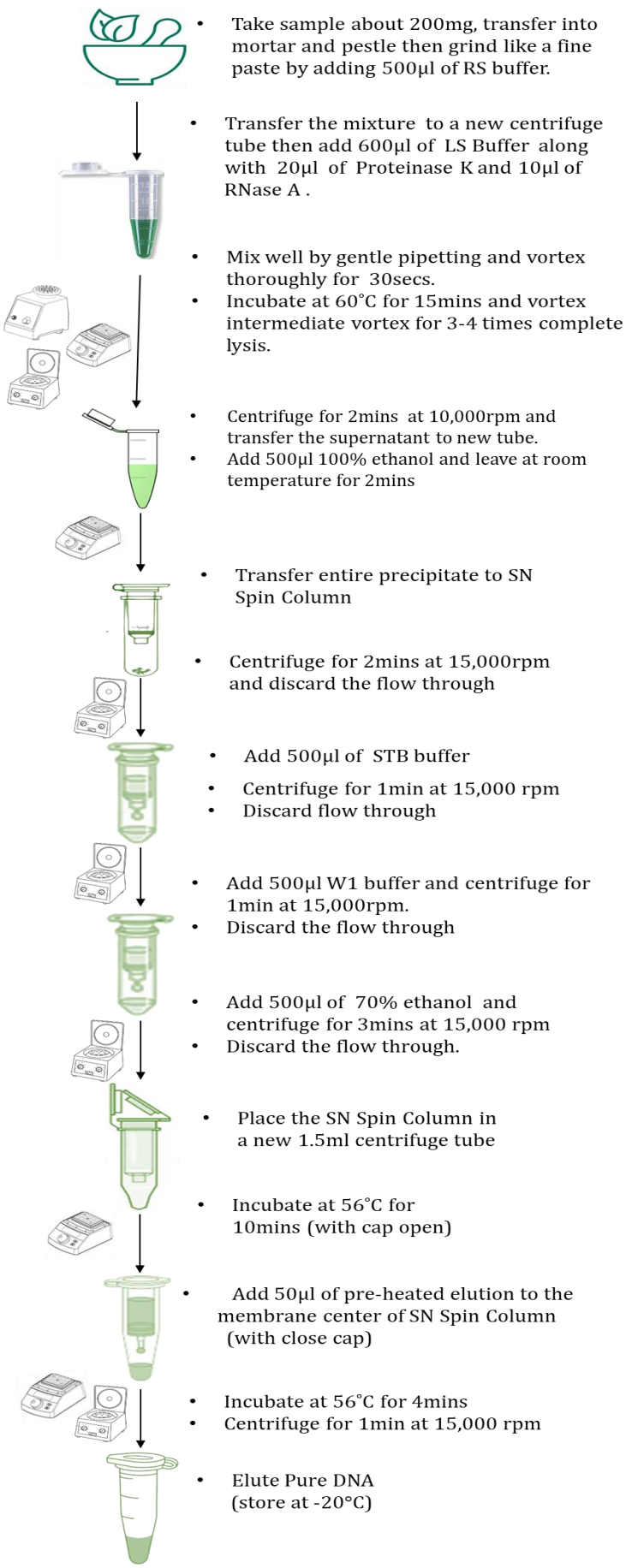
NOTE: Note : (W1 buffer concentration per reaction: W1 buffer - 300µl: 100% ETOH - 200µl)

9) Add 500µL of 70 % Ethanol Centrifuge the column at 15,000 rpm for 1min and centrifuge additional 1min.
10) Place the SN Spin Column in a new 1.5ml centrifuge tube and incubate for 10mins at 56˚C. (with cap open). This step is very important to remove the residual of ethanol.

NOTE: Elution should be pre-heated for 10mins before adding into the SN Spin Column.

11) Add 50µl of pre-heated Elution to the membrane center of SN Spin Column and incubate for 4mins at 56˚C (with cap close).
12) Centrifuge the tube at 15,000rpm for 1min to elute pure DNA(store at -20°C)

FLOW CHART



- Take sample about 200mg, transfer into mortar and pestle then grind like a fine paste by adding 500µl of RS buffer.
- Transfer the mixture to a new centrifuge tube then add 600µl of LS Buffer along with 20µl of Proteinase K and 10µl of RNase A .
- Mix well by gentle pipetting and vortex thoroughly for 30secs.
- Incubate at 60°C for 15mins and vortex intermediate vortex for 3-4 times complete lysis.
- Centrifuge for 2mins at 10,000rpm and transfer the supernatant to new tube.
- Add 500µl 100% ethanol and leave at room temperature for 2mins
- Transfer entire precipitate to SN Spin Column
- Centrifuge for 2mins at 15,000rpm and discard the flow through
- Add 500µl of STB buffer
- Centrifuge for 1min at 15,000 rpm
- Discard flow through
- Add 500µl W1 buffer and centrifuge for 1min at 15,000rpm.
- Discard the flow through
- Add 500µl of 70% ethanol and centrifuge for 3mins at 15,000 rpm
- Discard the flow through.
- Place the SN Spin Column in a new 1.5ml centrifuge tube
- Incubate at 56°C for 10mins (with cap open)
- Add 50µl of pre-heated elution to the membrane center of SN Spin Column (with close cap)
- Incubate at 56°C for 4mins
- Centrifuge for 1min at 15,000 rpm
- Elute Pure DNA (store at -20°C)

TROUBLE SHOOT

Problems	Possible reasons	Solutions
Low or none recovery of DNA fragment	Weigh 200mg of PLANT/FEED sample	If product is more, then separate it into multiple tubes.
	Elution DNA fragment is not efficient	Make sure the pH of Elution Buffer or ddH_20 is between 7.5-8.0.
Poor Performan ce in the downstrea m applicatio ns	Salt residue remains in eluted DNA	make sure that elution has been completely absorbed by the column
	Ethanol residue remains in eluted DNA	Do discard the flow- through after washing with W1 Buffer and centrifuge for an additional 1min.

IMPORTANT NOTES:

a) Buffer provided in this kit contains irritants. Wear gloves and lab coat when handling these buffers.

b) Add required volume of ethanol (96-100%) to wash Buffer before use.

c) Centrifugation steps are done by a micro centrifuge capable of the speed at 15,000rpm.

d) Check STB buffer for salt precipitation before use. Re-dissolve the precipitated salt by warming it in 37°C

e) Fresh TBE electrophoresis buffer is recommended on electrophoresis for experiments requiring high purity.