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A NEW GENERATION COMPANY

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SnLs
A NEW GENERATION COMPANY

SN Plant/Feed RNA extraction Kit

Magnetic Bead Based

Innovation Speaks

SN PLANT/FEED RNA EXTRACTION KIT
(Magnetic Bead - Based)

DESCRIPTION:

SN PLANT/FEED RNA Extraction Kit is designed for preparation of high-quality of total RNA from a wide range of PLANT/FEED samples. RNA in the whole homogeneity is selectively absorbed on the Magnetic Bead and other impurities are washed away . This kit provides a simple and efficient way to purify RNA.

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 Magnetic-Bead	250µl	2.5ml	5ml

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 DNase shipped in lyophilized form, upon receiving resuspend with Nuclease free water (5Rxn:50µl, 50Rxn:500µl, 100Rxn: 1ml)
- **Proteinase –K and DNase , 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	Mag-Bead
Recommended Input Amount :200mg of PLANT/FEED sample	
Binding Capacity : ~20-30µg genomic RNA	
Elution Volume : 50µl	
Purity: A260/280 - 1.8±0.1, A260/230 - 2.0±0.1	
Compatible Downstream Applications : PCR, Cloning, Next generation sequencing etc.	
Expected Yield : depending upon 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. Transfer the above mixture to 1.5 ml centrifuge tube using a clean spatula.
2) Add 600µl of LS buffer into the sample along with 20µl of Proteinase K and 10µl of DNase . Vortex for 1min.
3) Incubate at 60°C for 15mins with intermittent vortexing for 3-4 times to lysed the cells. Centrifuge the tube for 2mins at 10,000rpm, then transfer the supernatant to a fresh centrifuge tube.
4) Add 500µl of 100% ethanol to the supernatant, keep the tube for 2 mins at room temperature, and observe precipitation.
5) Transfer the complete precipitate to a fresh centrifuge tube, add 50µl of SN Magnetic bead and 500µl of STB buffer, mix well by vortexing for 30secs then keep at room temperature for 10mins.
6) Place the tube upon the magnetic rack (let the bead attracts towards magnet). Then remove the supernatant without disturbing the bead.
7) Add 500µl of W1 buffer mix well by vortexing for 30secs then keep at room temperature for 10mins.

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

8) Repeat the step (6)

9) Add 500µl of 70% ethanol, mix well by vortexing for 30secs then keep at room temperature for 5mins.

Repeat the step (6)

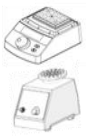
10) Incubate the tube at 56˚C for 10mins (with cap open).

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

11) Add 50µl of pre-heated Elution to the above tube, then vortex for 30secs and incubate at 56˚C for 4mins (with cap close)
12) Vortex for 10secs and then Centrifuge the tube at 15,000rpm for 2mins to Elute pure RNA (store at -20°C)

FLOW CHART:

- Weigh 200mg of plant sample and transfer into the mortar and pestle, add 500µl of RS buffer grind it like a paste.



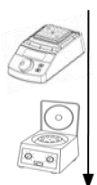
- Transfer into centrifuge tube and add 600µl of LS buffer along with 20µl of Proteinase K and 10µl of DNase.
- Mix well by vortexing 30 seconds and Incubate at 60˚C for 15mins

- Then centrifuge tube for 2mins at 10,000rpm.
- Then add 500µl of 100% ETOH, and leave at room temperature for 2mins. Transfer entire precipitate to a fresh centrifuge tube.

- Add 500µl STB Buffer along with 50µl of SN magnetic bead, mix well and vortex thoroughly for 30secs
- Allow to stand for 10 mins at room temperature.
- Place the tube upon the magnetic rack, it attracts bead and separates. Discard the supernatant without distrubing bead.

- Add 500µl W1 buffer and mix well by gentle pipetting few secs . Allow to stand for 10mins at room temperature.
- Place the tube upon the magnetic rack, it attracts bead and separates. Discard the supernatant without distrubing bead.

- Add 500µl of 70% ethanol and mix well by gentle pipetting few secs . Allow to stand for 5mins at room temperature.
- Place the tube upon the magnetic rack, it attracts bead and separates.
- Discard the supernatant without distrubing bead.



- Incubate at 56˚C for 10mins for ethanol evaporation (with cap open)

- Add 50µl of pre-heated Elution Buffer to the tube vortex few seconds (with cap close). Incubate at 56˚C for 4mins
- Centrifuge for 2mins at 15,000 rpm

- Pure RNA (store at -20°C)

TROUBLE SHOOTING:

Problems	Possible reasons	Solutions
Low or none recovery of RNA fragment	Weigh 200mg of PLANT/FEED sample	If product is more, then separate it into multiple tubes.
	Elution of RNA fragment is not efficient	Make sure the pH of Elution Buffer or ddH_20 is between 7.5-8.0
		Make sure that the elution solution has been observed by the magnetic beads.
		Preheat the elution solution at 56°C before use.
Poor Performance in the downstream applications	Salt residue remains in eluted RNA	
	Ethanol residue remains in eluted RNA	washing step is done with w1 buffer

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 11,000 ~ 15,000 rpm.
d) Fresh TBE electrophoresis buffer is recommended on electrophoresis for experiments requiring high purity.
e) Then excising the agarose gel, make sure to shorten the time of exposure to ultraviolet irradiation.