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**SHANKARANARAYANA
LIFE SCIENCES**
A NEW GENERATION COMPANY

SN RNA CATCHER
Soil RNA
Extraction Kit
(Magnetic Bead - Based)



SN SOIL RNA EXTRACTION KIT

(Magnetic Bead-Based)

DESCRIPTION:

SN Soil RNA Extraction Kit is designed for preparation of high-quality total RNA from a wide range of Soil samples. The preparation is based on magnetic bead based technology for binding RNA in high-salt and elution in low-salt buffer. This kit provides a simple and efficient way to elute pure RNA in minimum steps

CONTENTS OF KIT:

Sl. No	Components	Volume		
		5 Rxn	50 Rxn	100 Rxn
1	RS Buffer	1.5ml	15ml	30ml
2	LS Buffer	1.5ml	15ml	30ml
3	STB Buffer	2.5ml	25ml	50ml
4	W1 Buffer	2ml	20ml	40ml
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: 500µl, 50Rxn: 5ml, and 100Rxn: 10ml) 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** and **Carrier-RNA** shipped in lyophilized form, upon receiving resuspend with Nuclease free water (5Rxn:50µl, 50Rxn:500µl, 100Rxn: 1ml)
- ❖ **Proteinase K/Carrier-RNA/DNase** are store at -20°C either in liquid form or Lyophilized form

REQUIRED MATERIALS NOT PROVIDED :

- ✓ 100% Ethanol
- ✓ Dry bath
- ✓ 1.5ml Centrifuge tubes
- ✓ micro centrifuge
- ✓ Vortex
- ✓ Magnetic rack

STORAGE / SHIPPING:

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

SPECIFICATIONS:

Principle	Mag-Bead
Recommended Input Amount: 200mg Soil Sample	
Elution Volume : 50 µl	
Purity: A260/280 - 1.8±0.1	
Compatible Downstream Application: PCR, qPCR, Sequencing	
Expected Yield : depending upon the sample.	

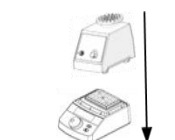
PROCEDURE:

- 1) Take 200mg of soil sample and add 300µl of RS buffer to a provided glass Bead tube and vortex thoroughly for 15mins.
- 2) Centrifuge the mixture at 3,000rpm for 4 mins.
- 3) Transfer the supernatant to a fresh tube. Add 300µl of LS Buffer along with 20µl of Proteinase K, 10µl DNase and 10µl of Carrier-RNA, Vortex for 30secs.
- 4) Incubate at 56°C for 15mins.
- 5) Add 500µl of 100% ethanol to the lysate and incubate for 2mins at room temperature to observe the precipitate.
- 6) Pipette out the precipitate and transfer to a fresh centrifuge tube.
- 7) Then add 500µl of STB buffer along with 50µl of SN Magnetic bead, mix well by vortexing 30 secs then keep at room temperature for 10mins.
- 8) Place the tube upon the magnetic rack (let the bead attracts towards magnet). Then remove the supernatant without disturbing the bead.
- 9) 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 -400µl: 100% ETOH -100µl)
- 10) Repeat the step(8).
- 11) Incubate the centrifuge tube at 56°C for 10mins (with cap open).
NOTE: Elution should be pre-heated for 10mins before adding into the tube.
- 12) Add 50µl of pre-heated Elution to the above tube, then vortex for 10secs and incubate at 56°C for 4mins (with close cap)
- 13) Vortex for 10secs and then Centrifuge the tube at 15,000rpm for 2mins to elute RNA (store at -20°)

FLOW CHART:



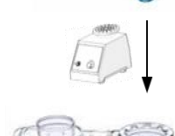
- Take 200mg of soil sample and add 300µl of RS buffer to a provided Bead tube and vortex thoroughly for 15mins.



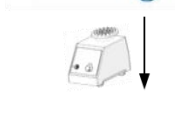
- Centrifuge the mixture at 3,000rpm for 4 mins.
- Transfer the supernatant to a fresh tube. Add 300µL of LS Buffer along with 20µL of Proteinase K, 10µL DNase and 10µl of Carrier-RNA. Vortex for 30secs.



- Incubate at 56°C for 15mins.
- Then add 500µl of ethanol and leave at room temperature for 2mins. The precipitate will form.
- Transfer the precipitate to a fresh centrifuge tube.



- Add 500µl of 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.



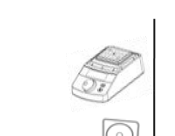
- Place the centrifuge upon the magnetic rack, it attracts bead and separates.
- Discard the supernant without distrubing the bead.



- Add 500µl W1 buffer and mix well by gentle pipetting 30secs .
- Allow to stand for 10mins at Room temperature



- Place the centrifuge upon the magnetic rack, it attracts bead and separates.
- Discard the supernant without distrubing the bead.



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



- Add 50µl of pre-heated Elution Buffer to the tube vortex 10 secs (with close cap).

- Incubate at 56°C for 4mins
- Vortex for 10 secs and centrifuge for 2mins at 15,000 rpm



- Elute Pure RNA(Store at -20°C)

TROUBLE SHOOTING:

Problems	Possible reasons	Solutions
Low or none recovery of RNA fragment	Weigh 200mg of soil 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 ₂ O is between 7.5-8.0.
Poor Performance in the downstream applications		Make sure that the elution solution has been observed by the magnetic beads.
	Salt residue remains in eluted RNA	Preheat the elution solution to 56°C before use.
	Ethanol residue remains in eluted RNA	washing step is done with W1 Buffer.

IMPORTANT NOTES:

- Ensure that the ethanol has been added in the wash buffer.
- Buffer provided in this kit contains irritants. Wear gloves and lab coat when handling these buffers.
- Add required volume of ethanol (96-100%) to W1 Buffer before use.
- Check stabilization buffer for salt precipitation before use. Re-dissolve the precipitated salt by warming it in 37°C.
- Fresh TAE electrophoresis buffer is recommended on electrophoresis for experiments requiring high purity.
- When excising the agarose gel, make sure to shorten time of exposure to ultraviolet irradiation.