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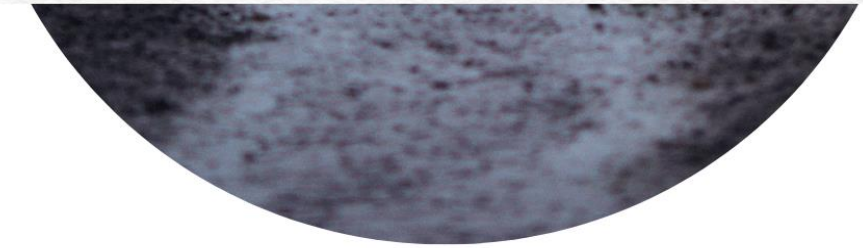
SN DNA CATCHER

# Soil DNA Extraction Kit

(Column Based)



INNOVATION SPEAKS

# SN SOIL DNA EXTRACTION KIT

(Column Based)

## DESCRIPTION:

SN SOIL DNA Extraction Kit provides an accurate, easy to use & rapid method to isolate high quality DNA from various soil samples. The preparation is based on a silica-membrane technology for binding DNA in high-salt and elution in low-salt buffer. This kit provides a simple and efficient way to elute pure DNA 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 | 2.5 ml | 25ml | 50ml |
| 4 | W1 Buffer | 2ml | 20ml | 40ml |
| 5 | Elution Buffer | 250µl | 2.5ml | 5ml |
| 6 | SN Spin Column & Collection tube | 5 No. | 50 No. | 100 No. |

NOTE: Preparation for first use after receiving the kit (Add 100% Ethanol to Wash Buffer) (10Rxn: 500µl, 50Rxn: 5ml, and 100Rxn: 5ml) 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) and store at -20°C.
- If **RNase A and Lysozyme** shipped in lyophilized form, upon receiving resuspend with Nuclease free water (5Rxn:50µl, 50Rxn:500µl, 100Rxn: 1ml) and store at -20°C.

**❖ Proteinase K/Lysozyme/RNase A 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

## STORAGE / SHIPPING:

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

## SPECIFICATIONS:

| Principle | Spin Column |
|---|---|
| Recommended Input Amount: 200mg Soil Sample | |
| Elution Volume : 50µI Recommended | |
| Purity: A260/280 - 1.8±0.1 | |
| Compatible Down8treem Application: PCR, qPCR, Sequencing | |
| Expected Yield : ~ 20µg | |

## PROCEDURE:

1) Take 200mg of soil sample and add 300µl of RS buffer to a provided Bead tube and vortex thoroughly for 15mins.
2) The mixture was centrifuge 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 RNase A and 10µl of Lysozyme. Vortex for 30secs.
4) Incubate at 56°C for 15mins.
5) Add 500µl of 100% ethanol and keep the tube for 2 mins at room temperature.
6) Transfer the complete precipitate to the SN Spin Column and leave at room temperature for 1min
7) Centrifuge at 15,000rpm for 1min and discard the flow-through in the collection tube.
8) Add 500µl of STB buffer to the SN Spin Column and centrifuge at 15,000rpm for 1min and discard the flow-through in the collection tube.
9) Add 500µl of W1 buffer to the SN Spin Column and centrifuge at 15,000 rpm for 1min and discard the flow-through in the collection tube.

**Note : (W1 buffer concentration per reaction: W1 buffer -400µl: 100% ETOH -100µl)**

10) Centrifuge the empty column at 15,000rpm for additional 1min.
11) Place the SN Spin Column in a new 1.5ml centrifuge tube and incubate for 10mins at 56˚C. (with cap open).

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

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

## FLOW CHART:

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

- Then centrifuge the tube for 4mins at 3,000rpm and then transfer the supernatant to fresh centrifuge tube

- Add 300µl LS Buffer + 20µl Proteinase K, 10µl of RNase A and 10µl of lysozyme

- Mix well by vortexing for 30secs.
- Incubate at 56˚C for 15min

- Add 500µl of 100% ethanol Incubate at room temperature for 2mins and observe precipitation
- Transfer entire precipitate to SN Spin Column and leave at room temperature for 1min.

- Centrifuge for 1min at 15,000rpm
- Discard flow through

- Add 500µl of STB buffer

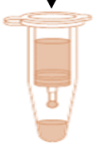
- Centrifuge for 1min at 15,000rpm
- Discard flow through

- Add 500µl W1 buffer

- Centrifuge for 1min at 15,000rpm
- Discard flow through
- Centrifuge for additional 1min at 15,000rpm

- Place the SN Spin Column in a new 1.5ml Eppendorf tube

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



- Add 50µl of pre-heated Elution Buffer to the membrane center of SN Spin Column (close cap)

- Incubate at 56˚C for 4mins
- Centrifuge for 1min at 15,000 rpm

- Pure DNA(store at-20°C)

## TROUBLE SHOOTING:

| Problems | Possible reasons | Solutions |
|---|---|---|
| Low or none recovery of DNA fragment | Weigh 200mg of soil sample | If product is more, then separate it into multiple tubes. |
| | Elution of DNA 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 completely absorbed by the column Membrane before centrifugation. |
| | | Preheat the elution solution to 56°C before use. |
| Poor Performance in the downstream applications | Salt residue remains in eluted DNA | Wash the column twice with W1 Buffer. |
| | 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) Ensure that the ethanol has been added in the wash buffer.
b) Buffer provided in this kit contains irritants. Wear gloves and lab coat when handling these buffers.
c) Check stabilization buffer for salt precipitation before use. Re-dissolve the precipitated salt by warming it in 37°C.
d) Fresh TAE electrophoresis buffer is recommended on electrophoresis for experiments requiring high purity.
e) Centrifugation steps are done by a micro centrifuge capable of the speed at 11,000 ~ 15,000 rpm