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SN Plant/Feed DNA extraction Kit

Magnetic Bead Based

SN PLANT/FEED DNA EXTRACTION KIT

(Magnetic Bead - Based)

DESCRIPTION:

SN PLANT/FEED DNA Extraction Kit is designed for preparation of high-quality of total DNA from a wide range of PLANT/FEED samples. DNA 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 DNA in a size range between 100 bp to 10 kb.

CONTENTS OF KIT:

Sl. No	Components	5 Rxn	Volume 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 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

Recommended Input Amount :200m	ng of
PLANT/FEED sample	

Binding Capacity: ~20-30μg genomic DNA

Elution Volume: 50µl

Purity: A260/280 - 1.8±0.1, A260/230 - 2.0±0.1

Mag-Bead

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 $600\mu l$ of LS buffer into the sample along with $20\mu l$ of Proteinase K and $10\mu l$ of RNase A . 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 $50\mu l$ of SN Magnetic bead and $500\mu l$ 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 DNA (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 RNase A.
- 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 10min s 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 DNA (store at -20°C)

TROUBLE SHOOTING:

Problems	Possible reasons	Solutions
Low or none recovery of DNA fragment	of	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 ₂ 0 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	Salt residue remains in eluted DNA	
downstream applications	Ethanol residue remains in eluted DNA	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.