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SuperSoil - Soil DNA Extraction

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Protocol status: Working

We use this protocol and it's working

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Abstract

SuperSoil - Soil DNA Extraction

Guidelines

Keep all solutions at room temperature

Materials

1. Bead (ball mill)

	A	B
	Zirconia Balls	150 μm : 500 μm = 1 : 2

2. Solution SD1 (Chaotropic)

	A	B	C
		(w/w)	Price
	Sodium thiocyanate	5%	1400/250 g
	Na ₂ HPO ₄	2.5%	1350/500 g
	pH	~8.4	

3. Solution SD2

	A	B	C
		(w/w)	Price
	AlCl ₃ · 6H ₂ O	1.5%	900/500 g
	Ammonium acetate	25%	820/ 500 g
	pH (Adjust with acetic acid)	6.3	

4. Solution SD3 (Chaotropic)

	A	B	C
		(w/w)	Price
	Guanidinium thiocyanate	65%	1800/250 g
	pH	~7.0	

5. Solution SEA (Wash buffer 1)

	A	B	C
		(w/w)	Price



	A	B	C
	Ethanol	50%	1000/4000 mL
	Isopropanol	10%	150/ 500 g
	Guanidine hydrochloride	30%	2900/1000 g

6.Solution S5 (Wash buffer 2)

	A	B	C
		(w/w)	Price
	Ethanol	70%	1000/4000 mL

7.Solution S6 (Elution buffer)

	A	B	C
		(w/w)	Price
	Tris Base	0.12%	3300/1000 g
	pH (Adjust with HCl)	8	

Safety warnings

! Some of the chemicals in this protocol may produce hazardous gases if mixed with bleach.



Lysis

- 1 Add **following materials** to 1.8 ml centrifuge tube
 1. 250 mg soil
 2. 1.5 g Bead
 3. 800 μ l SD1

- 2 **Vortex** for  00:10:00 at max speed

10m

Note

Vortex horizontally and ensure the cap is tightly sealed

- 3  14000 rpm, 00:01:00

1m

- 4 **Transfer the supernatant** to a clean 1.5 ml or 2 ml tube

Note

Expect 500–600 μ l

Remove inhibitor

1m

- 5 Add **200 μ l SD2**

**Note**

White precipitate forms.

If too much AlCl_3 (SD2) is added, it will cause all the DNA to be precipitated along with the inhibitory substances.

See Mustafa (2017) for more info.

Citation

Irfan Mustafa, Hadiatullah and Sustiyah (Invalid date)

. Removal of humic acid from peat soils by using AlCl_3 prior to DNA extraction.
AIP Conference Proceedings.

doi.org/10.1063/1.4983434

LINK

6 **Vortex** for  00:00:05 at max speed

5s

7  14000 rpm, 00:01:00

1m

Note

Don't worry if the supernatant is yellow.

8 **Transfer the supernatant** to a clean 1.5 ml or 2 ml tube

Note

Expect 500–600 μl

Bind DNA to silica membrane

2m 5s

9 Add **600 μl SD3**



10 **Vortex** for  00:00:05 at max speed

5s

11 **Transfer 650 ul lysate** to spin column

12  14000 rpm, 00:01:00 , discard flow-through

1m

13 **Transfer remaining lysate** to spin column

Wash silica membrane

1m

14 Add **500 ul SEA** to spin column

15  14000 rpm, 00:01:00 , discard flow-through

1m

16 Add **500 ul S5** to spin column

17  14000 rpm, 00:01:00 , discard flow-through

1m

18  14000 rpm, 00:01:00 , Centrifuge again to remove remaining ethanol (S5) , discard flow-through

1m

Elution

19 Add **50–100 ul S6**