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# UptiDNAPure DNA Purification Kit for Plant

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## Product Description

**Catalog number :** UPS54751 25 Purif.      UPS54761 50 Purif.      UPS54771 100 Purif.

**Description :** This kit has been tested in the following materials:

- \* Leaves
- \* Seeds
- \* Roots
- \* Flour
- \* Cooked material (boiled, sterilised, canned, etc.)
- \* Lyophilised
- \* Soya milk
- \* Fruits

### Reagents included in the kit

- Buffer B. Store at RT. If a precipitate is observed, heat at 50°C
- Proteinase K. Store at 4°C
- Solution I. Store at 4°C
- Silica Matrix. Store at 4°C. Never freeze or store under 2°C
- Wash Solution. Store at 4°C
- RNase A. Store at 4°C

### Reagents not included in the kit

- $\beta$ -mercaptoethanol. Store at 4°C
- Chloroform:isoamylalcohol (24:1). Store at 4°C
- Elution Buffer (TE or double-distilled water).

## Instructions for Use

### Standard Protocol

1. Homogenise sample (100-800 mg), either using liquid nitrogen or by mechanical homogenisation. If necessary, add 1 ml of Lysis Buffer (step 2).
2. Prepare Lysis Buffer by adding 15  $\mu$ l  $\beta$ -mercaptoethanol and 20  $\mu$ l Proteinase K (10 mg/ml) to 1 ml of Buffer B. Add 1 ml to homogenised sample and resuspend.
3. Incubate 1 hour at 65 °C. If maximum yield is required, increase incubation time to 3 hours. Alternatively, sample can be incubated at 37 °C O/N.
4. Centrifuge at 13000 rpm in a minifuge<sup>1</sup> for 15 min. Collect supernatant.
5. [OPTIONAL STEP. Add 100  $\mu$ g RNase I (10 mg/ml) (recommended for samples with high RNA content, like fresh fruits and seeds).
6. Incubate 1 hour at 37 °C.
7. Incubate on ice for 2 min. Add 0.7 volumes of chloroform:isoamy lalcohol (24:1). Mix well for 30 sec using vortex.
8. Centrifuge at 13000 rpm in a minifuge for 10 min, until phases separate.
9. Collect aqueous phase, without disturbing the interphase.

For any question,  
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10. Add 1 volume of Solution I. Mix thoroughly.
11. Add 80 µl of Silica Matrix (mix by pipetting before and after addition to the sample). No vortex.
12. Incubate 10 min at RT, mixing occasionally.
13. Centrifuge at 10000 rpm for 3 min in a minifuge.
14. Discard supernatant.
15. Add 700 µl cold Wash Solution (4°C). Resuspend by pipetting<sup>2</sup>.
16. Centrifuge at 10000 rpm for 3 min.
17. Repeat steps 15 and 16 (recommended).
18. Discard as much Wash Solution as possible. Spin Silica Matrix pellets for 30 sec at maximum speed, and drain off Wash Solution traces. Let air-dry for 10 min.
19. Add 150 µl of Elution Buffer (TE or double-distilled water)<sup>3</sup> preheated at 65 °C. Resuspend thoroughly.
20. Incubate 15 min at 60-65 °C, mixing occasionally. Incubation times may vary, depending on desired yield.
21. Centrifuge at maximum speed for 5 min in a bench-top centrifuge, and **immediately** proceed to the next step.
22. Collect eluate, without disturbing the matrix. <sup>4,5</sup>.
23. Quantify DNA in agarose electrophoresis. For amplification experiments, 50-100 ng are recommended. For Southern blot, and in order to reduce the eluate volume, it can be precipitated by using 1/10 vol of AcNa 3 M + 2 vol of cold ethanol.

<sup>1</sup> Centrifugation times and speed have been optimised for minifuges (i.e. Beckman Microfuge Lite., Heraeus Biofuge. 13, and similar devices).

<sup>2</sup> Pipetting is best performed with 100-1000 µl tips. Smaller tips may cause DNA shearing.

<sup>3</sup> Elution Buffer must have a pH above 7.0.

<sup>4</sup> If matrix has been carried over, spin vial for 1 min.

<sup>5</sup> For the maximum yield, repeat steps 20-22 with 50 µl of Elution Buffer.

*For invitro diagnostic use only/Pour usage diagnostique uniquement.*