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## UptiDNAPure for bacteria

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### Product Description

**Cat.Number:** UPS54521 (25 rxns), UPS54531 (50 rxns), UPS54541 (100 rxns)  
**Name :** UptiDNAPure Genomic DNA Purification kit - from Gram positive and Gram negative bacterial cultures

**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

#### Protocol 1 - Gram negative bacteria

1. Centrifuge for 5 min at 13000 rpm<sup>1</sup> 2 ml of culture in a sterile 2 ml vial. Discard supernatant. Repeat operation with 2 ml more, until a visible pellet is obtained (depending on the yield required). Discard supernatant.
2. Resuspend pellet in 1 ml TE.
3. Centrifuge 5 min at 13000 rpm. Discard supernatant.
4. Repeat steps 2 y 3.
5. Prepare Lysis Buffer by adding 15  $\mu$ l of  $\beta$ -mercaptoethanol and 20  $\mu$ l of Proteinase K (10 mg/ml) to 1 ml of Buffer B. Add 1 ml to the homogenised sample and resuspend.
6. Incubate at least for 30 min at 65 °C. If maximum yield is required, increase incubation time to 3 hours. Alternatively, sample can be incubated at 37 °C O/N.
7. Centrifuge at 13000 rpm in a minifuge<sup>1</sup> for 10 min. Collect supernatant.
8. Keep samples 5 min at room temperature. Then add 5  $\mu$ l of RNase A (10 mg/ml).
9. Incubate 30 min at 37 °C while constantly shaking.
10. Chill samples on ice. Add 0.7 volumes of chloroform:isoamylalcohol (24:1). Vortex vigorously for 30 sec.
11. Centrifuge at 13000 rpm in a minifuge for 10-15 min, until phases separate.
12. Collect the aqueous phase, carefully not to carry off the interphase.
13. Add 1 volume of Solution I. Mix thoroughly.
14. Add 80  $\mu$ l of Silica Matrix (mix by pipetting before and after addition to the sample). No vortex.
15. Incubate 10 min at RT, mixing occasionally.
16. Centrifuge at 10000 rpm for 3 min in a minifuge.
17. Discard supernatant.

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18. Add 700 µl cold Wash Solution (4°C). Resuspend by pipetting<sup>2</sup>.
19. Centrifuge at 10000 rpm for 3 min.
20. Repeat steps 18 and 19.
21. Discard supernatant. Let the pellet air-dry for 10 min.
22. Add 150 µl of Elution Buffer (TE or double-distilled water)<sup>3</sup> preheated at 65 °C. Resuspend thoroughly.
23. Incubate 15 min at 65 °C, mixing occasionally. Incubation times may vary, depending on desired yield.
24. Centrifuge at 10000 rpm for 3 min.
25. Collect the eluate, without disturbing the matrix.<sup>4,5</sup>
26. Quantify DNA at 260 nm or by agarose gel electrophoresis. For amplification experiments, 10-25 ng are recommended. For Southern blot, DNA can be concentrated by using 1/10 vol 3 M AcNa + 2 vol chilled ethanol.

<sup>1</sup> Centrifugation times and speed have been optimised for minifuges (i.e. Beckman Microfuge Lite™, Heraeus Biofuge™ 13, and similar). Other centrifuges may require different centrifugation times. Please contact our Technical Dpt. ([interbiotech@interchim.com](mailto:interbiotech@interchim.com)).

<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 traces have been carried over, spin for 1 min at maximum speed and transfer to new tubes.

<sup>5</sup> For maximum yields, repeat steps 22-25 with 50 µl of Elution Buffer.

## Protocol 2 - Gram positive bacteria (including cocci)

1. Centrifuge for 10 min at 13000 rpm<sup>1</sup> 2 ml of culture in a sterile 2 ml vial. Discard supernatant. Repeat operation with 2 ml more, until a visible pellet is obtained (depending on the yield required). Discard supernatant
2. Resuspend pellet in 1 ml TE.
3. Centrifuge 10 min at 13000 rpm. Discard supernatant.
4. Repeat steps 2 y 3.
5. Add 200 µl 10 mM Tris -10 mM glucose (pH 8.0)-lysozime (0.4 mg/ml final concentration).
6. Incubate 30 min at RT.
7. Follow with step 5 from protocol 1.

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