mi-Bacterial Genomic DNA Isolation Kit

For highly pure and rapid purification of genomic DNA from bacteria

Cat. No mi-BD100
[100 Preparations]

This kit is for research purposes only.
Not for use in diagnostic procedures.
For in vitro use only.

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Introduction
The mi-Bacterial Genomic DNA Kit is used to quickly and efficiently isolate high molecular weight genomic DNA (50-100kb) from bacteria cells. This kit is based on the efficient release of genomic DNA by using a special cell lysis buffer. A rapid separation of genomic DNA from proteins, polysaccharides and lipids by selective adsorption of DNA to the silica membrane becomes possible. The isolated DNA can be used for all molecular biology applications (RFLP, PCR, Southern Blotting, etc). This method is fast, safe and simple for DNA preparation from different sources of samples.

Kit Contents	**mi-Bacterial Genomic DNA Kit**
Preparation	100 rxn /kit
Cell Lysis Buffer	35 ml
PPT Buffer	15 ml
Column Binding Buffer	65 ml (NOTE: Ethanol has been added)
Column Wash Buffer	25 ml (NOTE: Add 100 ml pure ethanol up to a final volume of 125 ml prior to use.)
RNAse	dried powder (store at –20°C)
Spin column	100
Collection tube	100

Required Equipment
Microcentrifuge (13,000 rpm or 12,000 x g)
Vortexer
Microcentrifuge tubes
Distilled water (pH 7-8) or TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)

If you use gram positive bacteria:
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- Lysostaphin (20mg/ml)
- Lysozyme (20mg/ml)

Kit Storage
Store dried RNAse at –20°C upon kit arrival. Store solved RNAse at -20°C.
At room temperature for 6 months.

Precautions
See MSDS on our homepage (www.mymetabion.com).

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Protocol for the purification of DNA from bacteria

Note: Before start, please make sure
• To have completed the "Column Wash Buffer" by adding 100ml of pure ethanol (99.9%) before first use.
• To solve the dried RNAase in 300 µl water. Store the stock solution (10 mg/ml) at –20°C.
• All centrifugation steps are 13,000 rpm. Remember to orient tubes with hinges pointing straight out from center of the centrifuge rotor for all centrifugation steps.

1. Spin cells at 13,000 rpm (12,000 x g) for 1 min (1.5 ml bacterial culture).

2. For Gram-positive bacteria:
Resuspend cell pellet in 500 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) by vortexing.
Add 3 µl of each lytic enzyme which are not included in the kit [lysostaphin (20 mg/ml) and lysozyme (20 mg/ml)] to the sample.
Incubate at 37°C for 30 min to 1 hr.
Spin for 2 min at 13,000 rpm (12,000 x g) and discard the supernatant.
Add 300 µl of Cell Lysis Buffer and resuspend the pellet.
Incubate at 80°C for 5 min.

   For Gram-negative bacteria (E.coli…):
Add 300 µl of Cell Lysis Buffer and resuspend the pellet.
Incubate at 80°C for 5 min.

3. Cool the sample to room temperature. Pipette 3 µl of RNAse A (10 mg/ml) solution to the 1.5 ml tube and incubate at 37°C for 15 min.

4. Add 100 µl of PPT Buffer and vortex for 20 sec.
Incubate the sample on ice for 5 min.

5. Spin at 13,000 rpm (12,000 x g) for 10 min (room temperature).

6. Transfer the supernatant to a new 1.5 ml tube. Add 600 µl of Column Binding Buffer without wetting the rim and mix by vortexing.

7. Set the spin column into the collection tube.
Transfer 650 µl of the sample mix (prepared in step 6) to a spin column.

8. Spin at 13,000 rpm (12,000 x g) for 2 min.
The liquid will flow through the spin column membrane leaving the genomic DNA bound to the filter membrane.

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9. Remove the spin column from the collection tube and discard the flow through. Place the spin column in the same collection tube. Add the residual mixture (prepared in step 6) to a spin column, and spin at 13,000 rpm (12,000 x g) for 2 min.

10. Remove the spin column from the collection tube and discard the flow through. Place the spin column in the same collection tube.
Add 750 µl of Column Wash Buffer without wetting rim.
(Make sure to have completed the "Column Wash Buffer" by adding 100 ml pure ethanol before first use.)
Spin at 13,000 rpm (12,000 x g) for 2 min.

11. Remove the spin column from the collection tube and discard the flow through. Replace the spin column in the same collection tube.
Add 250 µl of Column Wash Buffer without wetting rim.
Spin at 13,000 rpm (12,000 x g) for 2 min.
[Residual wash buffer in the silica membrane may cause problems in downstream applications. In these cases, spin again at full speed (13,000 rpm for 1 min].

12. Place the spin column in a clean 1.5 ml microcentrifuge tube (not included), and discard the collection tube containing the filtrate.
Add 50 -100 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or distilled water. Incubate the spin column with TE buffer or distilled water (pH 7-8) at room temperature (15 -25°C) for 1 min and spin at 13,000 rpm (12,000 x g) for 1 min.
[Incubating the spin column loaded with TE buffer or distilled water for 5 min at room temperature before centrifugation generally increases DNA yield. The elution efficiency will decrease when using elution buffer with pH < 7.0].

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Hints and Troubleshooting

Concentrating the DNA

If your final elution volume is too diluted for your purposes, add 1.5 µl of 5 M NaCl and mix. Then add 70 µl of 100% cold ethanol. Mix and spin at 13,000 rpm (12,000 x g) for 5 min at 4°C. Decant all liquids. You can dry residual ethanol in a speed vac, desiccator or with ambient air. Resuspend precipitated DNA in desired volume.

Incomplete cell lysis

Extend lytic enzyme reaction time.
Incubating the spin column loaded with TE buffer or distilled water for 1-5 min at room temperature before centrifugation generally increases DNA yield. The elution efficiency will decrease when using elution buffer with pH <7.0.

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