DATA SHEET

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Biotecnologia ao seu alcance

# Bacteria DNA Preparation Kit

Spin column based genomic DNA purification from bacteria.

| Cat. Nº. | Amount |
|---|---|
| ☐ DPK-116S | 50 preparations |
| ☐ DPK-116L | 250 preparations |

**Shipping:**
Shipped at ambient temperature

**Storage Conditions:**
Store at ambient temperature (except Enzyme Mix and Proteinase K - store at - 20 °C)

**Shelf life:**
36 months

**For in vitro use only!**

**Kit Contents:**

- Cell Resuspension Solution
- Lysis Buffer
- Binding Buffer
- Enzyme Mix (before use, add Cell Ressuspention Solution as indicated on the tube and store at -20 °C)
- Proteinase K (before use, add double distilled water as indicated on the tube - store at -20 °C)
- Activation Buffer
- Washing Buffer (before use, add 96-99 % Ethanol as indicated on the tube)
- Elution Buffer
- Spin Columns and 2 ml Collection Tubes

**Additional Materials Required:**

- 96-99% Ethanol
- Ultra-Pure water
- 1.5 or 2.0 mL microtubes

**Applications:**

The obtained DNA is suitable for a variety of applications, including PCR amplifications, digestion with restriction enzymes and membrane hybridizations. Before start, add the following components as indicated on the respective bottle/tube:

Not included in the kit:

- double distilled water to Proteinase K
- 96-99 % Ethanol to the Washing Buffer

Included in the kit:

- Cell Ressuspention Solution to Enzyme Mix

**Description:**

The spin column based Bacteria and Plants tissue DNA Preparation Kit is designed for rapid and pure isolation of total DNA from bacteria Gram(+) or Gram(-). The spin column based method completely removes PCR inhibitors such as divalent cations and proteins resulting in a high purity preparation of genomic DNA. There is no use of phenol or chloroform, handling is safe and does not produce any harmful waste.

**PREPARATION PROCEDURE:**

For S pack (50 preps): Before start, add 500 µl dd-water to the Proteinase K tube, 1,5 ml of Cell ressuspention solution to Enzyme Mix tube and 44 ml 96-99 % Ethanol to Washing Buffer bottle.

For L pack (250 preps): Before start, add 500 µl dd-water to each Proteinase K tube, 1,5 ml of Cell ressuspention solution to each Enzyme Mix tube and 208 ml 96-99 % Ethanol to Washing Buffer bottle.

| Buffer | DPK-116S 50 preps | DPK-116L 250 preps |
|---|---|---|
| Lysis Buffer | 16 ml | 80 ml |
| Binding Buffer | 16 ml | 80 ml |
| Enzyme Mix | 2 microtubes | 10 microtubes |
| Proteinase K (10 mg/mL) | 5 mg | 5 x 5 mg |
| Cell Ressuspention buffer | 3,2 ml | 16 ml |
| Activation Buffer | 6 ml | 30 ml |
| Washing Buffer | 11 ml<br>Add 44ml EtOH | 52 ml<br>Add 208 ml EtOH |
| Elution Buffer | 5 mL | 25 mL |

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## 1. PREPARATION FROM BACTERIA

It is essential to use the correct amount of starting material in order to obtain optimal DNA yield and purify. A maximum amount of $10^9$ bacteria cells can generally be processed. Overnight cultured bacteria cells can be processed. Cell pellets can be stored at -70 °C for several months.

### A. Cell Lysis

- Harvest maximum 1 ml of cultured bacteria cells by centrifugation at 10,000 g for 1 min
- Discard the supernatant and vigorously vortex.

**NOTE:** After discarding the supernatant, the remaining 10-50 µl of media broth doesn't affect the next purification step. The vigorous vortexing will resuspend the pelleted cells in the remaining media and help the cell lysis

- Add **50 µl of Enzyme Mix** and vortex vigorously for 30-60 sec.
- Incubate tube at 37 °C for 10 min.
- Add **300 µl of Lysis buffer**
- Add **8 µl of Proteinase K** and mix by pipetting.
- Incubate at 60 °C for 10 min and cool down on ice for 5 min.

### B. DNA Binding

- Add **300 µl Binding Buffer** and vortex briefly or mix by inverting
- Place the tube one ice for 5 min
- Centrifuge for 5 min at 10,000 g (repeat if the supernatant is not clear)

### C. Column Activation

- Place a spin column into a 2 ml collection tube
- Add **100 µl Activation Buffer** into the Spin Column
- Centrifuge at 10,000 g for 30 sec and immediately proceed to next step
- Discard the flow-through

### D. Column Loading:

- Transfer 600 µl of the supernatant from **section B. DNA Binding** into a clean1,5 ml tube.
- Add 200 µl of absolute ethanol and vortex vigorously.
- Pipet **400 µl of the mixture** directly into the Spin Column (to avoid overloading of the column).
- Centrifuge for 1 min at 10,000 g.
- Discard the flow-through.
- Pipet the remaining **400 µl of the mixture** into the Spin Column.
- Centrifuge for 1 min at 10,000 g.
- Discard the flow-through.

### E. Washing:

- Add **500 µl Washing Buffer** into spin column
- Centrifuge for 30 sec at 10,000 g
- Discard the flow-through

***Optional: Repeat Washing Step if highest purity is required***

### F. Remove residual Washing Buffer

- Centrifuge at 10,000 g for 2 min to remove residual Washing Buffer
- Discard the 2 ml Collection Tube
- Place the Spin Column into a new 1.5 ml microtube

### G. Elution of DNA:

- Add **40-50 µl Elution Buffer** into the center of the spin column
- Incubate at room temperature for 1 min
- Centrifuge at 10,000 g for 2 min. Discard the spin column
- Store DNA at 4 °C or -20 °C

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## Troubleshooting

| Problem | Cause | Suggestions |
|---|---|---|
| Low yield | Insufficient lysis | Reduce the amount of starting material. Prolong the lysis step. Shake the sample during incubation |
| | No Ethanol added to Washing Buffer | Make sure to add EtOH to the Washing Buffer before start |
| | DNA is sheared/degraded | Avoid excessive pipetting after lysis. Do not vortex after lysis |
| | DNA is not completely eluted | Elute in two steps. Increase the volume of Elution Buffer. Prolong the incubation of the Elution Buffer on the Spin Column to 5 min. Make sure the Elution Buffer is delivered onto the matrix and not on the wall of the Spin Column. |
| RNA contamination | No / not enough Enzyme Mix added | Make sure to add Enzyme Mix in the Cell Lysis step |
| Inhibition of downstream enzymatic reactions | Ethanol carryover | Make sure to remove residual Washing Buffer before elution. Remove the Spin Column carefully from the Collection Tube so that it does not come in contact with the flow-through. If in doubt, centrifuge again for 1 min. |