DATA SHEET

Jena Bioscience

Bacteria DNA Preparation - Column Kit

Spin column based genomic DNA purification from bacteria

Cat. No.	Amount
PP-214S	50 preparations
PP-214L	250 preparations

For general laboratory use.

Shipping: shipped at ambient temperature

Storage Conditions: store at ambient temperature

Shelf Life: 12 months

Applications:
The obtained DNA is suitable for a variety of applications, including real-time PCR, southern blot analysis, genotyping and discovery or validation of SNP/SSR markers.
Before start, add the following components (not included in the kit) as indicated on the respective bottle/tube:

- double distilled water to RNase A, Lysozyme and Proteinase K
- 96-99 % Ethanol to the Washing Buffer

Description:
The spin column based Bacteria DNA Preparation Kit is designed for rapid and high purity isolation of genomic DNA from Gram-positive and Gram-negative bacteria. 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.
Column based genomic DNA purification kits yield up to 30 µg DNA sized from 200 bp to 50 kb per preparation.

Content:

- **Resuspension Buffer**
- **Lysis Buffer**
- **Binding Buffer**
- **RNase A**
  before use, add double distilled water as indicated on the bottle - store at -20 °C
- **Lysozyme**
  before use, add double distilled water as indicated on the bottle - 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**

To be provided by you:
96-99 % Ethanol
Double distilled water
Microtubes 1.5 or 2.0 ml

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Preparation procedure:
For S pack (50 preps): Before start, add 500 µl dd-water to the Proteinase K tube, 125 µl dd-water to the Lysozyme tube, 150 µl dd-water to the RNase A tube and 48 ml 96-99 % Ethanol (not included in the kit) to the Washing Buffer bottle.
For L pack (250 preps): Before start, add 500 µl dd-water to each Proteinase K tube, 125 µl dd-water to each Lysozyme tube, 150 µl dd-water to each RNase A tube and 120 ml 96-99 % Ethanol (not included in the kit) to each Washing Buffer bottle.

Buffer	**PP-214S** 50 preps	**PP-214L** 250 preps
Resuspension Buffer	16 ml	80 ml
Lysis Buffer	16 ml	80 ml
Binding Buffer	16 ml	80 ml
RNase A (50 mg/ml)	7.5 mg	5x 7.5 mg
Lysozyme (100 mg/ml)	12.5 mg	5x 12.5 mg
Poteinase K (10 mg/ml)	5 mg	5x 5 mg
Activation Buffer	6 ml	30 ml
Washing Buffer	add 48 ml Ethanol (final volume 60 ml)	add 120 ml Ethanol to each bottle (final volume 150 ml each)
Elution Buffer	5 ml	25 ml

It is essential to use the correct amount of starting material in order to obtain optimal DNA yield and purify. A maximum amount of 10^8 bacteria cells can generally be processed. Overnight cultured bacteria cells can be processed. Cell pellets can be stored at -70 °C for several months.

1. Preparation from Gram-positive bacteria
A Cell Resuspension:
- Harvest 500 µl of cultured bacteria cells by centrifugation at 10,000 g for 1 min
- Discard the supernatant
- Resuspend the cell pellet in 300 µl of Resuspension Buffer
- Add 2 µl of Lysozyme Solution
- Mix well by inverting several times
- Incubate tube at 37 °C for 1 hour
- Centrifuge at 10,000 g for 1 min
- Discard the supernatant

B Cell Lysis:
- Add 300 µl Lysis Buffer and 2 µl RNase A to cell pellet
- Vortex vigorously for 30-60 sec
- Add 8 µl Proteinase K and mix by pipetting
- Incubate at 60 °C for 10 min and cool down for 5 min
- Add 300 µl Binding Buffer and vortex briefly
- Place the tube on ice for 5 min
- Centrifuge for 5 min at 10,000 g

C Column Activation [optional]:
- 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:
- Pipet the supernatant directly into the spin column
- Centrifuge for 1 min at 10,000 g
- Discard the flow-through

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

F Secondary Washing:
- Add 500 µl Washing Buffer into the spin column.
- Centrifuge for 30 sec at 10,000 g
- Discard the flow-through
- Centrifuge again at 10,000 g for 1 min to remove residual Washing Buffer
- Discard the 2 ml wash tube and place the column in the elution tube

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

2. Preparation from Gram-negative bacteria
A Cell Lysis:
- Harvest 500 µl of cultured bacteria cells by centrifugation at 10,000 g for 1 min
- Discard the supernatant
- Add 300 µl Lysis Buffer and 2 µl RNase A to cell pellet
- Vortex vigorously for 30-60 sec
- Add 8 µl Proteinase K and mix by pipetting
- Incubate at 60 °C for 10 min and cool down for 5 min
- Add 300 µl Binding Buffer and vortex briefly
- Place the tube on ice for 5 min
- Centrifuge for 5 min at 10,000 g

B Column Activation [optional]:
- Place a spin column into a 2 ml collection tube

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- 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

C Column Loading:

- Place a spin column into a 2 ml collection tube
- Pipet the lysate directly into the spin column
- Centrifuge for 1 min at 10,000 g
- Discard the flow-through

D Primary Washing:

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

E Secondary Washing:

- Add 500 µl Washing Buffer into the spin column
- Centrifuge for 30 sec at 10,000 g
- Discard the flow-through
- Centrifuge again at 10,000 g for 1 min to remove residual Washing Buffer
- Discard the 2 ml wash tube and place the column in the elution tube

F Elution of DNA:

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