DATA SHEET

Jena Bioscience

Blood-Animal-Plant DNA Preparation - Columns Kit

Spin column based genomic DNA purification from blood, animal and plant cells

Cat. No.	Amount
PP-213S	50 preparations
PP-213L	250 preparations

For *in vitro* use only!

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 and Proteinase K
- 96-99 % Ethanol to the Washing Buffer

Description:
The spin column based Blood-Animal-Plant DNA Preparation Kit is designed for rapid and high purity isolation of genomic DNA from whole blood, animal cells and plant tissue. 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:

- **Blood Lysis Buffer**
- **Lysis Buffer**
- **Binding Buffer**
- **RNase A**
  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, 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, 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-213S** 50 preps	**PP-213L** 250 preps
Blood Lysis Buffer	55 ml	2x 137 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
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

1. DNA Preparation from blood
A Sample Preparation:
- Mix 200 µl (1 volume) of whole human blood with 1 ml (5 volumes) of Blood Lysis Buffer in an appropriately sized tube
- Incubate for 10 min on ice
- Mix by vortexing briefly 2-3 times during incubation
- Centrifuge at 10,000 g for 10 min to pellet the white blood cells
- Remove or discard supernatant completely

B Cell Lysis:
- Add 300 µl Lysis Buffer and 2 µl RNase A to the 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 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 the next step
- Discard flow-through

D Column Loading:
- Pipet supernatant directly into the spin column
- Centrifuge for 1 min at 10,000 g
- Discard flow-through

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

F Secondary Washing:
- Add 500 µl Washing Buffer into the spin column
- Centrifuge for 30 sec at 10,000 g
- Discard flow-through
- Centrifuge again at 10,000 g for 1 min to remove residual Washing Buffer
- Discard the 2 ml wash tube and place column into 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 the eluted DNA at 4 °C or -20 °C

2. DNA Preparation from animal cells
It is essential to use the correct amount of starting material in order to obtain optimal DNA yield and purity. A maximum amount of 80 mg animal material can generally be processed. We recommend to start with no more than 80 mg fresh or frozen animal tissue. Tissues can be stored at -70 °C for several months. Quick-freeze tissues in liquid nitrogen and grind thoroughly with a mortar and pestle. Transfer tissue powder into a 1.5 ml microcentrifuge tube cooled with liquid nitrogen.

A Cell Lysis:
- Add 300 µl Lysis Buffer and 2 µl RNase A to the tissue powder
- Vortex vigorously for 30-60 sec
- Add 8 µl Proteinase K and mix by pipetting
- Incubate at 60 °C for 20 min and cool down for 5 min
- Add 300 µl Binding Buffer and vortex briefly
- Place the tube one 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
- Add 100 µl Activation Buffer into the Spin Column
- Centrifuge at 10,000 g for 30 sec and immediately proceed to the next step
- Discard flow-through

C Column Loading:

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- Pipet supernatant directly into the spin column
- Centrifuge for 1 min at 10,000 g
- Discard flow-through

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

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

3. DNA Preparation from plant tissue

It is essential to use the correct amount of starting material in order to obtain optimal DNA yield and purity. A maximum amount of 80 mg plant material can generally be processed. We recommend to start with no more than 80 mg fresh or frozen plant tissue. Tissues can be stored at -70 °C for several months. Quick-freeze tissues in liquid nitrogen and grind thoroughly with a mortar and pestle. Transfer tissue powder into a 1.5 ml microcentrifuge tube cooled with liquid nitrogen.

A Cell Lysis:
- Add 300 µl Lysis Buffer and 2 µl RNase A to the tissue powder
- Vortex vigorously for 30-60 sec
- Add 8 µl Proteinase K and mix by pipetting
- Incubate at 60 °C for 20 min and cool down for 5 min
- Add 300 µl Binding Buffer and vortex briefly
- Place the tube one ice for 5 min
- Centrifuge for 5 min at 10,000 g

B 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 the next step
- Discard flow-through

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

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

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