DATA SHEET

Jena Bioscience

Tissue DNA Preparation - Column Kit

DNA purification from human, animal and plant tissue with silica-membrane columns

Cat. No.	Amount
PP-236L	250 preparations
PP-236S	50 preparations

For *in vitro* use only!

Shipping: shipped at ambient temperature

Storage Conditions: store at ambient temperature

Shelf Life: 12 months

Applications:
The Animal-Plant DNA Preparation kit allows extraction of up to 10 µg of highly pure genomic DNA in a size range from 200 bp to 50 kb per preparation. 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.

Description:
Human-Animal-Plant DNA Preparation - Column Kit is designed for rapid and high purity isolation of genomic DNA from human tissue or cells, animal tissue or cells and plant tissue or cells, respectively. Column-based purification 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 harmful waste.

Content:

Component	**PP-236S** 50 preps	**PP-236L** 250 preps
Lysis Buffer	16 ml	80 ml
RNase A *store RNase A solution at -20°C*	7.5 mg **add 150 µl ultra pure water**	5 x 7.5 mg **add 150 µl ultra pure water to each tube**
Proteinase K *store Proteinase K solution at -20°C*	5 mg **add 500 µl ultra pure water**	5x 5 mg **add 500 µl ultra pure water to each tube**
Binding Buffer	16 ml	80 ml
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)
Elution Buffer	5 ml	25 ml
Spin Columns	50	250
Collection Tubes, 2 ml	50	250

To be provided by you
- 96-99 % Ethanol
- ultra-pure water
- microtubes 1.5 or 2.0 ml
- heating block or water bath at 60 °C

Before start, add the following components
(not included in the kit) as indicated on the respective bottle/tube:

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- ultra-pure water to RNase A and Proteinase K (store solved enzymes at -20°C)
- 96-99 % Ethanol to the Washing Buffer

DNA Preparation

It is essential to use the correct amount of starting material in order to obtain optimal DNA yield and purity. Start with ≤ 80 mg wet weight or ≤ 20 mg lyophilized tissue.
Tissue can be quickly frozen in liquid nitrogen to simplify grinding. Frozen tissue is stable for several months.

1) Sample Preparation

- Mash or grind tissue thoroughly with a mortar and pestle
- Transfer tissue material into a 1.5 ml micro-centrifuge tube

2) Cell Lysis

- Add 300 µl Lysis Buffer and 2 µl RNase A
- Vortex vigorously for 30 sec
- Incubate at 60 °C for 5 min
- Add 8 µl Proteinase K and mix by pipetting
- Incubate at 60 °C for 10 min
- Cool down to room temperature

3) DNA Binding

- Add 300 µl Binding Buffer and mix by inverting
- Place the tube one ice to cool down
- Centrifuge for 5 min at 10,000 g (repeat if the supernatant is not clear)
- Transfer the supernatant into a new tube
- Add 500 µl Isopropanol (not included in the kit)
- Mix by pipetting up and down

4) 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
- Discard flow-through

5) Column Loading

- Pipet supernatant from cell lysis directly into the Spin Column
- Centrifuge for 1 min at 10,000 g
- Discard the flow-through

6) Washing

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

Optional: repeat for better results in purity

7) 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

8) Elution of DNA

- Add 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
- DNA in Elution Buffer is stable at 4 °C or -20 °C for several months

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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 Ethanol 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.
	No/ not enough RNase added	Make sure to add RNase A in the Cell Lysis step.
RNA contamination	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.
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.