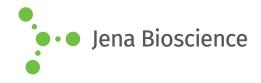
DATA SHEET





■ 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 | PP-236L |
|---|--|--|
| | 50 preps | 250 preps |
| Lysis Buffer | 16 ml | 80 ml |
| RNase A store RNase A solu- tion 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

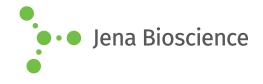
- 96-99 % Ethanol
- ultra-pure water
- microtubes 1.5 or 2.0 ml
- heating block or water bath at 60 $^{\circ}\text{C}$

Before start, add the following components

(not included in the kit) as indicated on the respective bottle/tube:



DATA SHEET





■ Tissue DNA Preparation - Column Kit

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

- 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 \leq 80 mg wet weight or \leq 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 μ l Lysis Buffer and 2 μ l 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

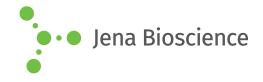
7) Remove residual Washing Buffer

- 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

DATA SHEET





Tissue DNA Preparation - Column Kit

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

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 contamina- tion | 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 en- zymatic 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. |