mi-Tissue Genomic DNA Isolation Kit

For highly pure and rapid purification of genomic DNA from tissue

Cat. No mi-TD100
[100 Preparations]

This kit is for research purposes only.
Not for use in diagnostic procedures.
For in vitro use only.

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Introduction

The mi-Tissue Genomic DNA Kit is used to quickly and efficiently isolate high molecular weight genomic DNA (50-100kb) from all kind of animal tissue and cell cultures (Maximum 100 mg wet weight or up to 20 mg dry weight (lyophilized) material). The yield range for 25-50 mg of mammalian tissue is 10 - 20 µg and for 10^6 culture cells 15-20 µg.
This kit is based on the efficient release of genomic DNA by using a special cell lysis buffer. A rapid separation of genomic DNA from proteins, polysaccharides and lipids by selective adsorption of DNA to the silica membrane becomes possible. The isolated DNA can be used for all molecular biology applications (RFLP, PCR, Southern Blotting, etc). This method is fast, safe and simple for DNA preparation from different sources of samples.

Kit Contents	mi-Tissue Genomic DNA Kit
Preparation	100 rxn /kit
Cell Lysis Buffer	35 ml
PPT Buffer	30 ml
Column Binding Buffer	65 ml (Note: Ethanol has been added)
Column Wash Buffer	25 ml (Note: Add 100 ml of pure ethanol (99,9%) up to a final volume of 125 ml prior to first use.)
RNAse	as powder (store at –20°C)
Spin column	100
Collection tube	100

Required Equipment
Microcentrifuge (13,000 rpm or 12,000 x g)
Vortexer
Microcentrifuge tubes
Homogenizer
PBS (if tissue culture cells are used)
Proteinase K (20mg / ml)
Distilled water (pH 7-8) or TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)

Kit Storage
Store dried RNAse at –20°C upon kit arrival. Store solved RNAse at -20°C.
At room temperature for 6 months.

Precautions
See MSDS on our homepage (www.mymetabion.com).

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Protocol for the purification of DNA from Tissue

Note: Before starting, please make sure…
• To have completed the Column Wash Buffer by adding 100 ml of pure ethanol before first use.
• To solve the dried RNAse in 300µl of water. Store the stock solution (10mg / ml) at –20°C.
• All centrifugation steps are at 13,000 rpm (12,000 x g). Remember to orient tubes with hinges pointing straight out from center of centrifuge rotor for all centrifugation steps.

1. For Animal Tissue:
   Homogenize up to 100 mg wet weight or up to 20 mg dry weight (lyophilized) material.
   [Methods to homogenize samples: Mortar and pestle in the presence of liquid nitrogen (all sample types), commercial homogenizers]
   Transfer the resulting powder to a 1.5 ml tube.

   For Tissue Culture Cells:
   Spin at 13,000 rpm (12,000 x g) for 30 min.
   Discard the supernatant and wash with PBS buffer.

2. Add 300 µl of Cell Lysis Buffer and mix thoroughly.
   Add 5 µl of Proteinase K (not included) (stock solution 20mg/ml) to the sample tube and mix thoroughly.

3. Incubate the sample at 55°C for 3 hours.

4. Cool the sample to room temperature. Add 3 µl of RNase A (10 mg/ml) to the 1.5 ml tube and incubate at 37°C for 15 -30 min.

5. Add 100 µl of PPT Buffer and vortex for 20 sec.
   Incubate the sample on ice for 5 min.

6. Spin at 13,000 rpm (12,000 x g) for 10 min at room temperature.

7. Transfer supernatant to a new 1.5 ml tube.
   Add 600 µl of Column Binding Buffer without wetting the rim and mix by vortexing.

8. Set a spin column into a collection tube. Transfer 650 µl of the sample (prepared in step 7) to the spin column.

9. Spin at 13,000 rpm (12,000 x g) for 2 min and discard the flow-through.
   The liquid will flow through the spin column membrane leaving the genomic DNA bound to the filter membrane. (Repeat steps 8 and 9 until all supernatant has passed through the column.)

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10. Remove the spin column from the collection tube and discard the flow through. Place the spin column in the same collection tube.
Add 750 µl of Column Wash Buffer without wetting the rim.
(Note: Make sure to have completed the Column Wash Buffer by adding 100 ml of pure ethanol before first use.)
Spin at 13,000 rpm (12,000 x g) for 2 min.

11. Remove the spin column from the collection tube and discard the flow through.
Re-place the spin column in the same collection tube.
Add 250 µl of Column Wash Buffer without wetting the rim.
Spin at 13,000 rpm (12,000 x g) for 2 min.
[Residual wash buffer in the silica membrane may cause problems in downstream applications. In these cases, spin again at full speed (more than 13,000 rpm for 1 min).]

12. Place the spin column in a clean 1.5 ml microcentrifuge tube (not included), and discard the collection tube containing the filtrate.
Elute by adding 50 - 100 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or distilled water (pH 7-8).
Incubate the spin column with TE buffer or distilled water at room temperature (15 -25°C) for 1 min, and spin at 13,000 rpm (12,000 x g) for 1 min.
[Incubating the spin column loaded with TE buffer or distilled water for 5 min at room temperature before spin generally increases DNA yield. The elution efficiency will decrease when using elution buffer with pH < 7.0].

The tissue genomic DNA is now ready to use!

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Hints and Troubleshooting

Concentrating the DNA

If your final volume is too diluted for your purpose, add 1.5 µl of 5 M NaCl and mix. Then add 70 µl of 100% cold ethanol. Mix and spin at 13,000 rpm (12,000 x g) for 5 min. Decant all liquid. Dry residual ethanol in a speed vac or desiccator or ambient air. Resuspend precipitated DNA in desired volume.

Incomplete cell lysis

The sample was not thoroughly mixed with Cell Lysis Buffer / Proteinase K. The mix has to be vortexed vigorously immediately after addition of Cell Lysis Buffer.

Proteinase K digestion is not optimal.

Never add Proteinase K directly to the Cell Lysis Buffer. Incubate for 10 min at 56°C.

Suboptimal elution of DNA from the spin column

Preheat the TE buffer or the distilled water to 70°C before the elution. Apply TE buffer or distilled water (pH 7-8) directly onto the centre of the silica membrane.

Poor DNA quality due to incomplete cell lysis

The sample was not thoroughly mixed with Cell Lysis Buffer / Proteinase K. The mixture has to be vortexed vigorously immediately after addition of Cell Lysis Buffer.

RNA contamination in the sample

If the DNA is desired without any RNA contamination, add 20 µl of an RNAse A solution (stock solution 20mg/ml) before adding the Cell Lysis Buffer.
Incubating the spin column loaded with TE buffer or distilled water for 1-5 min at room temperature before centrifugation generally increases the DNA yield. The elution efficiency will decrease when using elution buffer with pH < 7.0.

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