Genomic DNA Mini Kit (Tissue)

For research use only

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Sample	: up to 30 mg of tissue, up to 25 mg of paraffin-embedded tissue, buccal swab and up to 15 ml of amniotic fluid
Yield	: up to 50 µg
Format	: spin column
Operation time	: within 60 minutes
Elution volume	: 50-200 µl

Introduction

The Genomic DNA Mini Kit (Tissue) was designed specifically for purifying total DNA (including genomic, mitochondrial and viral DNA) from a variety of animal tissue, paraffin-embedded tissue, buccal swab and amniotic fluid. The provided micropestle can efficiently homogenize tissue samples to shorten the time in the Lysis Step. Proteinase K and chaotropic salt are used to lyse cells and degrade protein, allowing DNA to be easily bound by the glass fiber matrix of the spin column (1). Once any contaminants have been removed, using a Wash Buffer (containing ethanol), the purified DNA is eluted by a low salt Elution Buffer or TE. The entire procedure can be completed within 1 hour without phenol/chloroform extraction or alcohol precipitation. The expected yield of genomic DNA is up to 50 µg and the purified DNA (with approximately 20-30 Kb) is suitable for use in PCR or other enzymatic reactions.

Quality Control

The quality of the Genomic DNA Mini Kit (Tissue) is tested on a lot-to-lot basis by isolating genomic DNA from a 20 mg mouse liver sample. The Purified DNA (more than 10 µg with an A260/A280 ratio of 1.7 - 1.9) is quantified with a spectrophotometer and checked by electrophoresis.

Kit Contents

Name	GT004	GT050	GT100	GT300
GT Buffer	3 ml	30 ml	60 ml	155 ml
GBT Buffer	4 ml	40 ml	60 ml	155 ml
W1 Buffer	2 ml	45 ml	45 ml	130 ml
Wash Buffer* (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	25 ml (100 ml)	50 ml (200 ml)
Elution Buffer	1 ml	30 ml	30 ml	75 ml
Proteinase K** (Add ddH_20)	1 mg (0.1 ml)	11 mg (1.1 ml)	11 mg x 2 (1.1 ml)	65 mg (6.5 ml)
GD Column	4 pcs	50 pcs	100 pcs	300 pcs
2 ml Collection Tube	8 pcs	100 pcs	200 pcs	600 pcs
Micropestle	4 pcs	50 pcs	100 pcs	300 pcs

Order Information

Product Name	Package Size	Cat. No.
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	GB100/300
Genomic DNA Midi Kit (Blood/Cultured Cell)	25 preps	GDI25
Genomic DNA Maxi Kit (Blood/Cultured Cell)	10/25 preps	GDM10/25
Genomic DNA Mini Kit (Tissue)	50/100/300 preps	GT050/100/300
Genomic DNA Mini Kit (Plant)	100 preps	GP100
Genomic DNA Maxi Kit (Plant)	10/25 preps	GPM10/25
96-Well Genomic DNA Kit	2/4/10 x 96 Wells	GBP02/04/10
96-Well Genomic DNA Kit (Plant)	2/4/10 x 96 Wells	GPP02/04/10
Vacuum Manifold (Accessories)	1 set	ZFV04

*Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).

**Add ddH_20 to prepare the Proteinase K (vortex to dissolve and spin down) and store at 4^oC (see the bottle label for volume).

Caution

GBT Buffer contains guanidine hydrochloride which is a harmful irritant. During operation, always wear a lab coat, disposable gloves, and protective goggles.

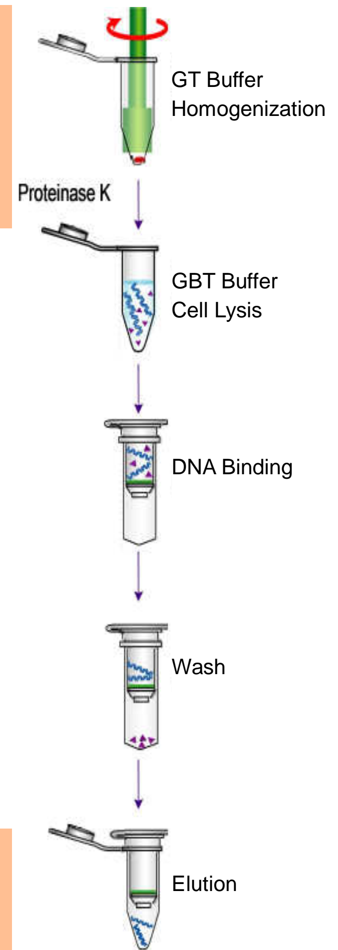
References

(1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

Genomic DNA Mini Kit (Tissue) Protocol

- Add ddH_2O to prepare the Proteinase K (vortex to dissolve and spin down) and store at 4ºC (see the bottle label for volume).
- Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
- Additional requirements: microcentrifuge tubes, absolute ethanol, RNase A (10 mg/ml), ddH_2O

Step	Procedure
Tissue Dissociation	• Cut up to 30 mg of animal tissue (or 0.5 cm of mouse tail) and transfer it to a 1.5 ml microcentrifuge tube. If the tissue has a higher number of cells (eg. spleen or liver), reduce the starting material to 10 mg. • Use the provided **Micropestle** to grind the tissue to a pulp. • Add **200 µl of GT Buffer** to the tube and continue to homogenize the sample tissue by grinding.
Step 2 Lysis	• Add **20 µl of Proteinase K** to the sample mixture and mix by vortex. • Incubate at 60ºC for 30 minutes to lyse the sample. During incubation, invert the tube every 5 minutes. • Add **200 µl of GBT Buffer** and mix by vortex for 5 seconds. • Incubate at 70ºC for 20 minutes or until the sample lysate is clear. During incubation, invert the tube every 5 minutes. At this time, preheat the required **Elution Buffer** (200 µl per sample) to 70ºC (for Step 5 DNA Elution). • If there is insoluble material present following incubation, centrifuge for 2 minutes at 14-16,000 x g and transfer the supernatant to a new 1.5 ml microcentrifuge tube. **Optional Step: RNA Degradation** If RNA free genomic DNA is required, perform this optional step. • Following 70ºC incubation, add 4 µl of RNase A (10 mg/ml) to the sample lysate and mix by vortex. • Incubate at room temperature for 5 minutes.
Step 3 DNA Binding	• Add 200 µl of absolute ethanol to the sample lysate and vortex immediately for 10 seconds. If precipitate appears, break it up by pipetting. • Place a **GD Column** in a **2 ml Collection Tube**. • Transfer all of the mixture (including any precipitate) to the **GD Column**. • Centrifuge at 14-16,000 x g for 2 minutes. • Discard the **2 ml Collection Tube** containing the flow-through and transfer the **GD Column** to a new **2 ml Collection Tube**.
Step 4 Wash	• Add **400 µl of W1 Buffer** to the **GD Column**. • Centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through and place the **GD Column** back in the **2 ml Collection Tube**. • Add **600 µl of Wash Buffer** (ethanol added) to the **GD Column**. • Centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through and place the **GD Column** back in the **2 ml Collection Tube**. • Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.
Step 5 DNA Elution	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl. • Transfer the dried **GD Column** to a clean 1.5 ml microcentrifuge tube. • Add **100 µl of preheated Elution Buffer** or TE to the center of the column matrix. • Let stand for 5 minutes or until the **Elution Buffer** or TE is absorbed by the matrix. • Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.



Genomic DNA Mini Kit (Tissue) Paraffin-Embedded Tissue Protocol

- Additional Requirements: xylene, absolute ethanol, microcentrifuge tube

Step	Procedure
Step 1 Sample Preparation	• Slice small sections (up to 25 mg) from blocks of paraffin-embedded tissue and transfer to a 1.5 ml microcentrifuge tube. • Add 1 ml of xylene to the tube. Vortex vigorously and incubate at room temperature for approximately 10 minutes. Vortex occasionally during incubation. • Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant. • Add 1 ml of absolute ethanol to wash the sample pellet and mix by inverting. • Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant. • Add 1 ml of absolute ethanol to wash the sample pellet again and mix by inverting. • Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant. • Open the tube and Incubate at 37ºC for 15 minutes to evaporate any ethanol residue. • Proceed with the Lysis Step of the Tissue Protocol.

Genomic DNA Mini Kit (Tissue) Buccal Swab Protocol

- Additional Requirements: Swab: cotton, DACRON or C.E.P. swabs, PBS (phosphate-buffered saline), absolute ethanol, microcentrifuge tube

Step	Procedure
Step 1 Sample Preparation	• Scrape the swab firmly against the inside of each cheek 6-7 times and air-dry the swab. (The person providing the sample should not eat or drink for at least 30 minutes prior to sample collection to avoid contamination.)
Step 2 Lysis	• Add **500 µl of GT Buffer** and **20 µl of Proteinase K** to a 1.5 ml microcentrifuge tube. • Place the buccal swab into the tube and incubate at 60ºC for 10 minutes. • Discard the swab and add **500 µl of GBT Buffer** to the lysate. • Vortex immediately and incubate at 60ºC for 10 minutes. • At this time, preheat the required **Elution Buffer** (200 µl per sample) in a 60ºC water bath (for Step 5 DNA Elution).
Step 3 DNA Binding	• Add 500 µl of absolute ethanol to the sample lysate and vortex immediately. • Place a **GD Column** in a **2 ml Collection Tube**. • Transfer 700 µl of the mixture to the **GD Column**. • Centrifuge at 14-16,000 x g for 1 minute. • Repeat the DNA Binding Step by transferring the remaining mixture to the **GD Column**. • Proceed with the Wash Step of the Tissue Protocol.

Genomic DNA Mini Kit (Tissue) Amniotic Fluid Protocol

- Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
- Additional requirements: centrifuge tube, microcentrifuge tubes, RNase A (10 mg/ml), absolute ethanol

Step	Procedure
Step 1 Cell Harvesting/ Prelysis	• Transfer approximately 10 ml (up to 15 ml) of amniotic fluid to a 15 ml centrifuge tube. • Centrifuge for 3 minutes at 14-16,000 x g and discard the supernatant.
Step 2 Cell Lysis	• Add **200 µl of GT Buffer** to resuspend the pellet and transfer the mixture to a 1.5 ml microcentrifuge tube. • Add **10 µl of Proteinase K (10 mg/ml)** to the sample mixture and mix by vortex. • Incubate at 60ºC for 30 minutes to lyse the sample. During incubation, invert the tube every 5 minutes. • Add **200 µl of GBT Buffer** to the 1.5 ml microcentrifuge tube and mix by vortex for 5 seconds. • Incubate at 70ºC for 20 minutes or until the sample lysate is clear. During incubation, invert the tube every 5 minutes. • At this time, preheat the required **Elution Buffer** (200 µl per sample) to 70ºC. (for Step 5 DNA Elution) **Optional Step: RNA Degradation** If RNA-free genomic DNA is required, perform this optional step. • Add 5 µl of RNase A (10 mg/ml) to the sample lysate following the 70ºC incubation and mix by vortex. • Incubate at room temperature for 5 minutes.

Step 3 DNA Binding	• Add 200 µl of absolute ethanol to the sample lysate and vortex immediately for 10 seconds. If precipitate appears, break it up by pipetting. • Place a **GD Column** in a **2 ml Collection Tube.** • Transfer all of the mixture (including any precipitate) to the **GD Column**. • Centrifuge at 14-16,000 x g for 1 minute. • Discard the **2 ml Collection Tube** containing the flow-through and place the **GD Column** in a new **2 ml Collection Tube**.
Step 4 Wash	• Add **400 µl of W1 Buffer** to the **GD Column**. • Centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through and place the **GD Column** back in the **2 ml Collection Tube**. • Add **600 µl of Wash Buffer** (ethanol added) to the **GD Column**. • Centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through and place the **GD Column** back in the **2 ml Collection Tube**. • Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.
Step 5 DNA Elution	Standard elution volume is 100 µl. If less sample volume is used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl. • Transfer the dried **GD Column** to a clean 1.5 ml microcentrifuge tube. • Add **100 µl of preheated Elution Buffer** or TE to the center of the column matrix. • Incubate the **GD Column** at 37ºC in an incubator for 10 minutes. • Centrifuge at 14-16,000 x g for 1 minute to elute the purified DNA.

Troubleshooting

Problem	Possible Reasons/Solution
Clogged Column	**Too much tissue was used** • If using more than 30 mg of tissue, separate into multiple tubes.
	Sample tissue was not lysed completely • Add additional Proteinase K and extend the incubation time in the Lysis Step. • Following the Lysis Step, centrifuge for 2 minutes at 14-16,000 x g to remove sample debris. Transfer the supernatant to a new microcentrifuge tube and proceed with the DNA Binding Step.
	Precipitate was formed at DNA Binding Step • Reduce the sample material. • Before loading the column, break up the precipitate in the ethanol added lysate.
Low Yield	**Sample tissue was not lysed completely** • Add additional Proteinase K and extend the incubation time in the Lysis Step.
	Column was clogged at DNA Binding Step • Following the Lysis Step, remove the insoluble debris by centrifugation. • Prior to loading the column, break up the precipitate in the ethanol added lysate.
	Incorrect DNA Elution Step • Ensure that the Elution Buffer or TE is added in the center of the GD Column matrix and is absorbed completely.
	Incomplete DNA Elution • Elute twice to increase the DNA recovery.
Eluted DNA does not perform well in downstream applications.	**Residual ethanol contamination** • Following the Wash Step, dry the GD Column with additional centrifugation at 14-16,000 x g for 5 minutes or incubate at 60ºC for 5 minutes.
	RNA contamination • Perform optional RNA Degradation step.
	Protein contamination • Reduce the sample amount.
	Genomic DNA was degraded • Use fresh samples or freeze fresh samples in liquid nitrogen immediately and store at -80ºC.