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IBI SCIENTIFIC

INSTRUCTION MANUAL

Genomic DNA Mini Kit (Tissue)

IB47220
IB47221
IB47222

Sample: up to 30 mg of tissue (tailsnips, liver, kidney, brain, adipose tissue, earpunches, insects etc.)

Yield: 10-20 µg (0.5 cm of mouse tail, 20 mg of mouse liver), 20-50 µg (20 mg of mouse kidney)

Format: spin column

Time: within 30 minutes

Elution Volume: 30-200 µl

Storage: dry at room temperature (15-25ºC)

Introduction

The Genomic DNA Mini Kit (Tissue) was designed specifically for purifying total DNA (including genomic, mitochondrial and viral DNA) from a variety of tissue and insect samples. 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. Once any contaminants have been removed, using a Wash Buffer (containing ethanol), the purified DNA is eluted by a low salt Elution Buffer, TE or water. The entire procedure can be completed without phenol/chloroform extraction or alcohol precipitation. The purified DNA (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 1.8-2.0) is quantified with a spectrophotometer and analyzed by electrophoresis.

Kit Contents

Component	IB47220	IB47221	IB47222
GT Buffer	3 ml	30 ml	75 ml
GBT Buffer	3 ml	40 ml	75 ml
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer** (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
Proteinase K** (Add ddH_2O)	1 mg (0.1 ml)	11 mg (1.1 ml)	65 mg (6.5 ml)
Elution Buffer	1 ml	30 ml	75 ml
GS Columns	4	50	300
2 ml Collection Tubes	8	100	600
Micropestle	4	50	300

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

** Add ddH_2O (see the bottle label for volume) to prepare Proteinase K (vortex to dissolve and spin down) and store at 4ºC.

Order Information

Product	Package Size	Catalogue #
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	IB47201/202
Genomic DNA Mini Kit (Tissue)	50/300 preps	IB47221/222
gMAX Mini Kit (Blood/Tissue)	100/300 preps	IB47281/282
Genomic DNA Mini Kit (Plant)	100 preps	IB47230

Product	Package Size	Catalogue #
gSWAB Mini Genomic DNA Ki	100/300 preps	IB47276/277
gBAC Mini DNA Bacteria Kit	100/300 preps	IB47291/292
gYEAST Genomic DNA Kit	100/300 preps	IB47266/267
96 Well Blood Genomic DNA Extraction Kit	4/10 x 96 preps	IB47251/252
gPURE Cell DNA Isolation Kit	100/1000 rxns	IB47431/432

Caution

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

Genomic DNA Mini Kit (Tissue) Functional Test Data

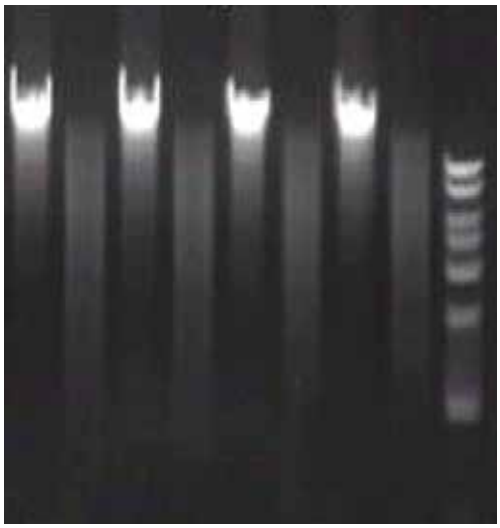
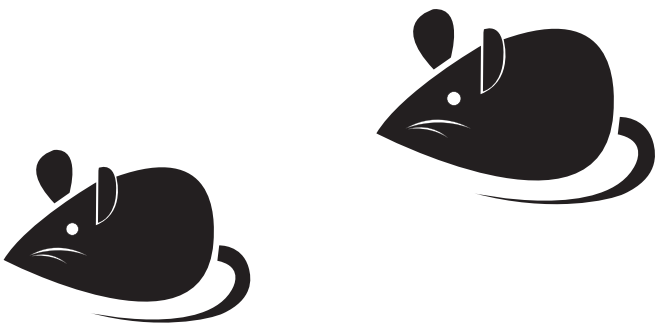


Figure 1. Genomic DNA from a variety of tissue samples was extracted using the Genomic DNA Mini Kit (Tissue). The purified genomic DNA (30-40 kb) was EcoRI digested and analyzed by electrophoresis on a 1% agarose gel.

1 = Mouse Liver
2 = Mouse Tail
3 = Fish Muscle
4 = Fruit Fly (Drosophila)
M = Geneaid 1 Kb DNA Ladder



Genomic DNA Mini Kit (Tissue) Protocol

IMPORTANT BEFORE USE!

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

Tissue Dissociation	• Cut up to 30 mg of animal tissue (or 0.5 cm of mouse tail) then transfer it to a 1.5 ml microcentrifuge tube. NOTE: If tissue has a higher number of cells (e.g. 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 homogenize the sample tissue by grinding. • Add 20 µl of Proteinase K to the sample mixture then shake vigorously and incubate at 60°C for 30 minutes. NOTE: During incubation, invert the tube every 5 minutes.
Step 1 Lysis	• Add 200 µl of GBT Buffer then shake vigorously for 5 seconds. • Incubate at 60°C for at least 20 minutes to ensure the lysate is clear. NOTE: During incubation, invert the tube every 5 minutes. If insoluble material is present following incubation, centrifuge for 2 minutes at 14-16,000 x g then transfer the supernatant to a new 1.5 ml microcentrifuge tube. At this time, preheat the required Elution Buffer (200 µl per sample) to 60°C (for Step 4 DNA Elution). Optional Step: RNA Degradation (If RNA free gDNA is required, perform this optional step) • Following 60°C incubation, add 4 µl of RNase A (10 mg/ml) to the sample lysate then shake vigorously. • Incubate at room temperature for 5 minutes.
Step 2 DNA Binding	• Add 200 µl of absolute ethanol to the lysate then immediately shake vigorously for 10 seconds. NOTE: If precipitate appears, break it up as much as possible with a pipette. • Place a GS Column in a 2 ml Collection Tube. • Transfer the mixture (including any precipitate) to the GS Column then centrifuge at 14-16,000 x g for 2 minutes. • Discard the 2 ml Collection Tube then transfer the GS Column to a new 2 ml Collection Tube.
Step 3 Wash	• Add 400 µl of W1 Buffer to the GS Column then centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through then place the GS Column back in the 2 ml Collection Tube. • Add 600 µl of Wash Buffer (make sure ethanol was added) to the GS Column. • Centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through then place the GS Column back in the 2 ml Collection Tube. • Centrifuge for 3 minutes at 14-16,000 x g to dry the column matrix.
Step 4 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 approx. 200 µl. • Transfer the dried GS Column to a clean 1.5 ml microcentrifuge tube. • Add 100 µl of pre-heated Elution Buffer or TE to the CENTER of the column matrix. • Let stand for at least 5 minutes to ensure the Elution Buffer or TE is completely absorbed. • Centrifuge at 14-16,000 x g for 30 seconds 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. • Following ethanol addition, break up any precipitate as much as possible prior to loading GS Column.
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 to the center of the GS 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 GS Column by centrifuge at 14-16,000 x g or incubate at 60°C for 5 minutes.
	RNA/Protein contamination • Perform optional RNA Degradation step/reduce the sample amount.
	Genomic DNA was degraded • Use fresh samples or freeze fresh samples in liquid nitrogen immediately and store at -80°C.

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