



vivantis
Nucleic Acid Extraction Kit HandBook

GF-1

**TISSUE DNA EXTRACTION
USER GUIDE (Version 4.1)**

Catalog No.

SAMPLE: 5 preps

GF-TD-050: 50 preps

GF-TD-100:100 preps

High Yield and Purity

Fast and Easy purification

Reliable and Reproducible

Eluted DNA ready for use in downstream applications

No toxic or organic-based extraction required

Introduction

The **GF-1 Tissue DNA Extraction Kit** is designed for rapid and efficient purification of genomic DNA from up to 5×10^6 cultured animal cells and various organs such as kidney, heart, lungs, brain, muscles, liver, spleen, etc without the need for precipitation or organic extractions. This kit uses a specially-treated glass filter membrane fixed into a column to efficiently bind DNA in the presence of high salt. This kit applies the principle of a mini-column spin technology and the use of optimized buffers to ensure that only DNA is isolated while cellular proteins, metabolites, salts and other low molecular weight impurities are removed during the subsequent washing steps. High-purity genomic DNA is eluted in water or low salt buffers and has a $A_{260/280}$ ratio between 1.7 and 1.9 making it ready to use in many routine molecular biology applications such as restriction enzyme digestion, Southern blotting, PCR, DNA fingerprinting and other manipulations.

Kit components

Product Catalog No.	5 Preps SAMPLE	50 Preps GF-TD-050	100 Preps GF-TD-100
Components			
GF-1 columns	5	50	100
Collection tubes	5	50	100
Tissue Lysis Buffer (Buffer TL)	1.5ml	15ml	30ml
Lysis Enhancer	0.1ml	1ml	2ml
Tissue Genomic DNA Binding Buffer (Buffer TB)	3.2ml	32ml	64ml
Wash Buffer (concentrate)*	2.4ml	24ml	2 x 24ml
Elution Buffer	1.5ml	10ml	20ml
Proteinase K*	0.11ml	1.05ml	2 x 1.05ml
Handbook	1	1	1

* Please refer to **Reconstitution of Solutions** and **Storage and Stability** before using this kit.

The **GF-1 Tissue DNA Extraction Kit** is available as 50 and 100 purifications per kit.
The reagents and materials provided with the kit are for research purposes only.

Note: The **GF-1 Tissue DNA Extraction Kit** is optimized to isolate up to 20 μ g of DNA from up to 5×10^6 cultured animal cells or 10 - 20mg of tissue samples. Tissue samples vary in the number of cells depending on age, type of tissue and origin. When processing samples, do not use more than the recommended starting material as excessive number of cells will overload the column. This would result in reduced yield and purity. We recommend weighing the tissue samples before starting to ensure optimum yield and purity is obtained. Liver and spleen are very high in protein and RNA content. Thus, when isolating genomic DNA from these sources, use only up to 15mg of the sample.

Additional Materials to be Supplied by User

Absolute Ethanol (>95%)

RNase A (DNase-free) (20mg/ml)

Phosphate Buffered Saline (PBS)

Reconstitution of Solutions

The bottle labeled **Wash Buffer** contains concentrated buffer which must be diluted with absolute ethanol (>95%) before use.

For **SAMPLE (5 preps)**,

Add **5.6ml** of absolute ethanol into the bottle labeled **Wash Buffer**.

For **GF-TD-050 (50 preps)**,

Add **56ml** of absolute ethanol into the bottle labeled **Wash Buffer**.

For **GF-TD-100 (100 preps)**,

Add **56ml** of absolute ethanol into one of the bottles labeled **Wash Buffer**.

Add **56ml** of absolute ethanol into the other bottle labeled **Wash Buffer** only prior to use.

Store **Wash Buffer** at room temperature with bottle capped tight after use.

Storage and Stability

Store solutions at 20°C - 30°C.

Proteinase K is stable for up to 1 year after delivery when stored at room temperature or 4°C. To prolong the lifetime of Proteinase K, storage at -20°C is recommended.

Kit components are guaranteed to be stable for 18 months from the date of manufacture **Buffer TB** may exhibit salt precipitation due to cold temperature. If this occurs, simply warm the bottle at 55°C - 65°C with occasional mixing until precipitate is completely dissolved.

Chemical Hazard

Buffer TB contains guanidine salts which can be harmful when in contact with skin or swallowed. Always wear gloves and practice standard safety precautions. Do NOT disinfect guanidine or extraction waste in solutions containing bleach or any other form of acid. To clean any items contaminated with the reagent, simply soak in detergent and water to remove all traces of guanidine before cleaning with bleach or acidic solutions.

Procedures

Reminder

- All steps are to be carried out at room temperature unless stated otherwise.
- **Wash Buffer** (concentrate) has to be diluted with absolute ethanol before use. Please refer to **Reconstitution of Solutions**.
- If precipitation forms in **Buffer TB**, incubate at 55°C - 65°C with occasional mixing until precipitate is completely dissolved.

Pre-set waterbath to 65°C.

Pre-heat **Elution Buffer** at 65°C.

A. DNA Extraction from Cultured Animal Cells

1. Centrifugation and resuspension

Pellet appropriate amount of cells (maximum 5×10^6) in a clean microcentrifuge tube by centrifugation at $800 \times g$ for 5 min at 4°C. Decant the supernatant. Add 200µl of PBS and resuspend completely by pipetting.

If frozen cells pellet is used, thaw the cells completely on ice before adding PBS.

2. Cells lysis

Add in 20µl of **Proteinase K** and 2µl of **Lysis Enhancer** to the sample and mix immediately. Add 200µl of **Buffer TB** and mix thoroughly by pulsed-vortexing. Incubate at 65°C for 10 min.

Proceed to Step 4.

B. DNA Extraction from Animal Tissue

1. Tissue preparation

Cut 10 - 20mg of tissue sample into small pieces with a clean scalpel.

The tissue sample can be ground into fine powder using liquid nitrogen with a pestle and mortar for more efficient lysis.

2. Tissue lysis

Add 250µl of **Buffer TL** and 20µl of **Proteinase K** to the sample. Mix thoroughly by pulsed vortexing to obtain a homogeneous solution. Add 12µl of **Lysis Enhancer** and mix immediately. Incubate at 65°C

for 1-3hr (or overnight if tissue mixture does not appear clear) in a shaking waterbath or mix occasionally during incubation to ensure thorough digestion of the sample.

*If tissue sample has not been reduced to small pieces, homogenize sample in **Buffer TL** with multiple strokes using a tube pestle. Solubilization of tissue sample varies between different tissue types. If insoluble materials still remain, extend incubation time or increase the amount of Proteinase K to ensure complete lysis.*

Optional: Removal of RNA

If RNA-free DNA is required, add 20µl of RNase A (DNase-Free, 20mg/ml). Mix and incubate at 37°C for 10 min.

3. Homogenization

Add 2 volumes (~560µl without RNase A treatment, ~600µl with RNase A treatment) of **Buffer TB** and mix thoroughly by pulsed-vortexing until a homogeneous solution is obtained. Incubate 10 min at 65°C.

4. Addition of ethanol

Add 200µl of absolute ethanol. Mix immediately and thoroughly by pulsed-vortexing to obtain a homogeneous solution.

Mix immediately to prevent any uneven precipitation of nucleic acid due to high local ethanol concentrations.

5. Loading to column

Transfer approximately 650µl of sample into a column assembled in a clean collection tube (provided). Centrifuge at 5,000 x g for 1 min. Discard flow through.

Repeat for the remaining sample from step 4.

6. Column washing

Wash the column with 650µl **Wash Buffer** and centrifuge at 5,000 x g for 1 min. Discard flow through. Repeat column washing once again.

Ensure that ethanol has been added into the Wash Buffer before use (refer to Reconstitution of Solutions).

7. Column drying

Centrifuge the column at 10,000 x g for 1 min to remove all traces of ethanol.

8. DNA elution

Place the column into a clean microcentrifuge tube. Add 200µl of preheated **Elution Buffer**, TE buffer or sterile water directly onto column membrane and stand at room temperature for 2 min. Centrifuge at 5,000 x g for 1 min to elute DNA. Store DNA at 4°C or -20°C.

Troubleshooting

Please note that by not adhering to the recommended protocols, unsatisfactory results related to yield and quality of DNA may occur. If problems arise, please refer to the following:

Problem	Possibility	Suggestions
Problem Low DNA yield	<i>Sample not thoroughly homogenized</i>	<i>Ensure that tissues are completely homogenized in Buffer TL</i>
	<i>Samples not fresh or not properly stored</i>	<i>For long term storage of tissues, keep at -70°C.</i>
	<i>Sample not lysed completely</i>	<i>Ensure that tissues are completely homogenized in Buffer TL, Proteinase K and Lysis Enhancer, mix sample frequently during incubation in the absence of a shaking waterbath.</i>
		<i>Ensure that cultured animal cells are completely homogenized in PBS, Proteinase K, Lysis Enhancer and Buffer TB, mix until homogeneous by pulsed-vortexing before incubation at 65°C.</i>
	<i>Proteinase K activity is decreased</i>	<i>For long term storage, ensure that Proteinase K is stored at -20°C.</i>
	<i>Low elution efficiency</i>	<i>Pre-heat Elution Buffer to 65°C-70°C before eluting DNA.</i>
	<i>Incubate column at room temperature for 2 min after addition of Elution Buffer.</i>	
	<i>Ensure that Elution Buffer used is a low salt buffer or water with a pH range of 7.0-8.5.</i>	

Problem	Possibility	Suggestions
	<i>Column clogged</i>	<i>Refer to Problems under 'Column clogged'.</i>
Low purity (A260/280)	<i>Incomplete protein denaturation</i>	<i>Use fresh Proteinase K and extend incubation time until lysate clears.</i>
	Proteinase K activity is decreased	<i>Refer to problem "Low DNA yield".</i>
Column clogged	<i>Overloading of column</i>	<i>Do not use more than recommended amounts of sample material. If any undigested material remains, spin to remove tissue lysate and transfer supernatant into a new microcentrifuge tube.</i>
DNA degradation/smearing	<i>DNA sheared during purification</i>	<i>After the addition of Buffer TL and Proteinase K, avoid vigorous mixing and pipetting. Use cut-off tip if lysate appeared viscous.</i>
	<i>Sample too old</i>	<i>DNA already degraded in old sample.</i>
	<i>Sample frozen and thawed repeatedly</i>	<i>Avoid repeated freeze-thaw cycles.</i>
Poor performance of eluted DNA in downstream applications	<i>Eluted DNA contains traces of ethanol</i>	<i>Ensure that the Column drying step is carried out prior to elution.</i>
	<i>TE buffer is used to elute DNA. EDTA in TE buffer may inhibit subsequent enzymatic reaction</i>	<i>Use Elution Buffer or water with a Ph range of 7.0 - 8.5.</i>

A. DNA Extraction from Cultured Animal Cells

Centrifugation and resuspension

Pellet cells at $800 \times g$ for 5 min. Add $200 \mu\text{l}$ of PBS and resuspend completely

Cell lysis

Add $20 \mu\text{l}$ of Proteinase K.
Add $2 \mu\text{l}$ of Lysis Enhancer.
Add $200 \mu\text{l}$ of Buffer TB. Mix by pulsed-vortexing

Proceed to Addition of ethanol

B. DNA Extraction from Animal Tissue

Tissue preparation

Cut tissue into small pieces or grind into fine powder in liquid nitrogen

Tissue lysis

Add $250 \mu\text{l}$ Buffer TL.
Add $20 \mu\text{l}$ Proteinase K.
Mix by pulsed-vortexing.
Add $12 \mu\text{l}$ Lysis Enhancer.
Incubate 65°C , 1 - 3 hr.

Optional: Removal of RNA

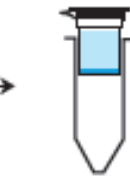
Add 20 I RNase A.
Incubate 37°C , 10 min.

Homogenization

Add 2 volumes Buffer TB and mix thoroughly.
Incubate 65°C , 10 min.

Addition of ethanol

Add $200 \mu\text{l}$ absolute ethanol and mix immediately.



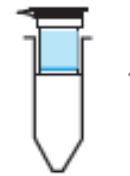
Loading to column

Transfer sample to column



Centrifuge

Discard flow through



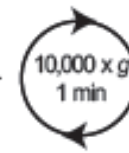
Column washing

Add $650 \mu\text{l}$ Wash Buffer



Centrifuge

Discard flow through
Repeat again washing step



Column drying



Elution

Transfer column to a new microcentrifuge tube.
Add $200 \mu\text{l}$ preheated Elution Buffer or water. Stand for 2 min.



Centrifuge

Store DNA at 4°C or -20°C