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 $5 \times 10^6$ 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**

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

* 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 20µg of DNA from up to $5 \times 10^6$ 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 x 10⁶) in a clean microcentrifuge tube by centrifugation at 800 x 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:

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possibility</th>
<th>Suggestions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Problem</td>
<td>Sample not thoroughly homogenized</td>
<td>Ensure that tissues are completely homogenized in <strong>Buffer TL</strong></td>
</tr>
<tr>
<td>Low DNA yield</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Samples not fresh or not properly stored</td>
<td>For long term storage of tissues, keep at -70°C.</td>
</tr>
<tr>
<td></td>
<td>Sample not lysed completely</td>
<td>Ensure that tissues are completely homogenized in <strong>Buffer TL</strong>, <strong>Proteinase K</strong> and <strong>Lysis Enhancer</strong>, mix sample frequently during incubation in the absence of a shaking waterbath.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ensure that cultured animal cells are completely homogenized in <strong>PBS</strong>, <strong>Proteinase K</strong>, <strong>Lysis Enhancer</strong> and <strong>Buffer TB</strong>, mix until homogeneous by pulsed-vortexing before incubation at 65°C.</td>
</tr>
<tr>
<td></td>
<td><strong>Proteinase K</strong> activity is decreased</td>
<td>For long term storage, ensure that <strong>Proteinase K</strong> is stored at -20°C.</td>
</tr>
<tr>
<td>Low elution efficiency</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-heat <strong>Elution Buffer</strong> to 65°C-70°C before eluting DNA.</td>
<td>Incubate column at room temperature for 2 min after addition of <strong>Elution Buffer</strong>.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ensure that Elution Buffer used is a low salt buffer or water with a pH range of 7.0-8.5.</td>
</tr>
<tr>
<td>Problem</td>
<td>Possibility</td>
<td>Suggestions</td>
</tr>
<tr>
<td>--------------------------------------</td>
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<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Low purity</td>
<td>Incomplete protein denaturation</td>
<td>Use fresh <strong>Proteinase K</strong> and extend incubation time until lysate clears.</td>
</tr>
<tr>
<td>Low purity</td>
<td><strong>Proteinase K</strong> activity is decreased</td>
<td>Refer to problem &quot;Low DNA yield&quot;.</td>
</tr>
<tr>
<td>Column clogged</td>
<td>Overloading of column</td>
<td>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.</td>
</tr>
<tr>
<td>DNA degradation/smearing</td>
<td>DNA sheared during purification</td>
<td>After the addition of <strong>Buffer TL</strong> and <strong>Proteinase K</strong>, avoid vigorous mixing and pipetting. Use cut-off tip if lysate appeared viscous.</td>
</tr>
<tr>
<td>DNA degradation/smearing</td>
<td>Sample too old</td>
<td>DNA already degraded in old sample.</td>
</tr>
<tr>
<td>DNA degradation/smearing</td>
<td>Sample frozen and thawed repeatedly</td>
<td>Avoid repeated freeze-thaw cycles.</td>
</tr>
<tr>
<td>Poor performance of eluted DNA</td>
<td>Eluted DNA contains traces of ethanol</td>
<td>Ensure that the Column drying step is carried out prior to elution.</td>
</tr>
<tr>
<td>Poor performance of eluted DNA</td>
<td>TE buffer is used to elute DNA. EDTA in TE buffer may inhibit subsequent enzymatic reaction</td>
<td>Use <strong>Elution Buffer</strong> or water with a Ph range of 7.0 - 8.5.</td>
</tr>
</tbody>
</table>
A. DNA Extraction from Cultured Animal Cells

Centrifugation and resuspension
Pellet cells at 800 x g for 5 min. Add 200 µl of PBS and resuspend completely

Cell lysis
Add 20 µl of Proteinase K. Add 2 µl of Lysis Enhancer. Add 200 µl 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

Optional: Removal of RNA
Add 20 µl 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 µl absolute ethanol and mix immediately.

Loading to column
Transfer sample to column

Centrifuge
Discard flow through

Column washing
Add 600 µl Wash Buffer

5,000 x g 1 min

Centrifuge
Discard flow through
Repeat again washing step

10,000 x g 1 min

Column drying
Transfer column to a new microcentrifuge tube. Add 200 µl preheated Elution Buffer or water. Stand for 2 min.

5,000 x g 1 min

Elution
Store DNA at 4°C or -20°C

Contact: info@vivanttechnologies.com