96 GF-1

96-WELL TISSUE DNA EXTRACTION KIT USER GUIDE
(Version 2.1)

Catalog No.

GF-96-T05: 96 x 5plate
GF-96-T10: 96 x 10plate

Yields up to 20µg of genomic DNA

Purification process takes less than 60 minutes after sample lysis

No organic-based extraction required

Highly pure genomic DNA ready to use for routine molecular biology applications

Introduction

The **GF-1 96-well Tissue DNA Extraction Kits** is designed for rapid and high-throughput purification of genomic DNA from animal tissues, for 96 samples simultaneously. The kit uses a specially-treated glass filter membrane fixed into a 96-well format plate to efficiently bind DNA in the presence of high salt. The use of optimized buffers ensures that only DNA is isolated while cellular proteins, metabolites, salt and other low molecular weight impurities are removed during the subsequent washing steps. DNA eluted in low salt buffers or water is ready to use in many routine molecular applications such as restriction enzyme digestion, PCR, Southern Blotting, DNA fingerprinting and other manipulations. The entire procedure can be done by centrifuge or on vacuum manifold.

Kit component

Product Catalog No.	5 x 96 GF-96-T05	10 x 96 GF-96-T10
Components		
GF-1 96-well DNA Binding Plate	5	10
Deep Well Collection Plate	10	20
96-well Storage Plate	5	10
Sealing Film	20	40
Caps for Storage Plate	60	120
Buffer PBS	200ml	2 x 200ml
Buffer ACL	200ml	2 x 200ml
Buffer AB	200ml	2 x 200ml
Wash Buffer (concentrate)*	4 x 48ml	8 x 48ml
Elution Buffer	50ml	2 x 50ml
Proteinase K	200mg	2 x 200mg
Handbook	1	1

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

The **GF-1 96-well Tissue DNA Extraction Kit** is available as 5 x 96 and 10 x 96 purifications per kit.
The reagents and materials provided with the kit are for research purposes only
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Additional Materials to be Supplied by User

Absolute Ethanol (>95%)
Xylene

Reconstitution of Solutions

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

For **GF-96-T05 (5 x 96) and GF-96-T10 (10 x 96),**

Add **192ml** of absolute ethanol into the bottle labeled **Wash Buffer.** Store **Wash Buffer** at room temperature with bottle capped tightly after use.

Add **12.5ml** nuclease-free water into the tube labeled **Proteinase K.** Mix well and store at -20°C for long term storage.

Storage and Stability

Store all solutions at 20°C – 30°C.
Store **Proteinase K** at 4°C. For long term storage, we recommend that users store the Proteinase K solution at -20°C and in small aliquots to avoid repeated freeze-thaw cycles.

Kit components are guaranteed to be stable for 18 months from the date of manufacture.
Buffer ACL and **Buffer AB** 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 ACL and **Buffer AB** contain 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 ACL** and **Buffer AB**, incubate at 55 - 65°C with occasional mixing until precipitate is completely dissolved.
- Users are recommended to use a multichannel pipette.
- For centrifugation based method, there is a minimum height requirement of 75mm for apparatus to hold the assembly of 96-Well DNA Binding Plate and Deep Well Collection Plate.

Pre-set incubator / oven to 55°C.
Pre-heat Elution Buffer to 65 - 70°C.

I. Animal Tissue

1. Sample lysis

Cut 20-30mg of animal tissue into small pieces with a clean scalpel or ground the sample in liquid nitrogen, and put it into a clean Deep Well Collection Plate.

Do not use more than 30mg of tissue as exceed starting materials will leads to lower yield. Do not use more than 15mg for tissues with very high number of cells, such as spleen. Avoid repeated freeze-thaw process on frozen samples.

Add 300µl of **Buffer ACL** and 20µl **Proteinase K** into each well of the deep well collection plate. Seal the deep well collection plate properly and firmly with the sealing film. Mix thoroughly by vortexing to obtain a homogenous solution. Incubate the collection plate at 55°C until the sample is completely lysed.

Lysis time varies depending on the tissue type, typically 1-3 hours, or can be lysed overnight. Vortex occasionally to disperse the samples. Lysate may appear viscous after incubation. If the lysate appeared gelatinous, prolong the incubation time.

2. Homogenization

Add 300µl of **Buffer AB** into the samples without wetting the rims of the wells. Seal the deep well collection plate properly and firmly with a new sealing film. Mix thoroughly by vortexing to obtain a homogenous solution. Centrifuge the collection plate briefly at 5700 x *g* to collect solution from the sealing film. Incubate at 55°C for 20mins until the sample lysate is clear.

Ensure that the deep well collection plate is sealed properly to avoid cross-contamination during shaking.

3. Please refer to Part A for Centrifugation Protocol
Please refer to Part B for Vacuum Protocol

II. Rodent Tail

1. Sample lysis

Cut 0.5-1cm from the end of tails into small pieces with a clean scalpel or ground the sample in liquid nitrogen, and put it into a clean Deep Well Collection Plate.
Add 300µl of **Buffer ACL** and 20µl **Proteinase K** into each well of the deep well collection plate. Seal the deep well collection plate properly and firmly with the sealing film. Mix thoroughly by vortexing to obtain a homogenous solution. Incubate the collection plate at 55°C overnight with rocking or several hours with occasional vortexing.

2. Homogenization

Add 300µl of **Buffer AB** into the samples without wetting the rims of the wells. Seal the deep well collection plate properly and firmly with a new sealing film. Mix thoroughly by vortexing to obtain a homogenous solution. Centrifuge the collection plate briefly at 5700 x *g* to collect solution from the sealing film. Incubate at 55°C for 20mins until the sample lysate is clear.
Ensure that the deep well collection plate is sealed properly to avoid cross-contamination during shaking.

3. Please refer to Part A for Centrifugation Protocol
Please refer to Part B for Vacuum Protocol

III. Cultured Animal Cells

1. Sample lysis

Pellet appropriate amount of cells (maximum 5 x 10^6) for 5mins at 5700 x *g* in a deep well collection plate. Decant the supernatant. Add 500µl of PBS and resuspend completely by pipetting. Wash the cells 2 times with PBS.

Add 300µl of **Buffer ACL** and 20µl **Proteinase K** into each well of the deep well collection plate. Seal the deep well collection plate properly and firmly with the sealing film. Mix thoroughly by vortexing to obtain a homogenous solution. Incubate the collection plate at 55°C for 10mins.

2. Homogenization

Add 300µl of **Buffer AB** into the samples without wetting the rims of the wells. Seal the deep well collection plate properly and firmly with a new sealing film. Mix thoroughly by vortexing to obtain a homogenous solution. Centrifuge the collection plate briefly at 5700 x *g* to collect solution from the sealing film. Incubate at 55°C for 10mins until the sample lysate is clear.
Ensure that the deep well collection plate is sealed properly to avoid cross-contamination during shaking.

3. Please refer to Part A for Centrifugation Protocol
Please refer to Part B for Vacuum Protocol

IV. Paraffin Tissue

1. Sample pre-treatment

Excise 25-30mg of paraffin tissue with a clean scalpel and put it into a clean deep well collection plate. Add 1.2ml xylene to the deep well collection plate. Seal the deep well collection plate properly and firmly with a new sealing film. Mix thoroughly by vortexing for 3 min to obtain a homogenous solution. Xylene is used to remove paraffin.

Centrifuge at 5700 x *g* for 5 mins at room temperature. Decant the supernatant. Add 1.2ml absolute ethanol (>98%) into the deep well collection plate. Seal and vortex gently for 1 min. stand at room temperature for 1 min. centrifuge at 5700 x *g* for 5 mins at room temperature. Decant the supernatant. Repeat this washing step once again.

Incubate the deep well collection plate at 37°C for 10-15mins to remove residual ethanol.

2. Sample lysis

Add 300µl of **Buffer ACL** into each well of the deep well collection plate. Seal the deep well collection plate properly and firmly with the sealing film. Mix thoroughly by vortexing to obtain a homogenous solution. Incubate the collection plate at 55°C for 10 mins.

3. Homogenization

Add 300µl of **Buffer AB** into the samples without wetting the rims of the wells. Seal the deep well collection plate properly and firmly with a new sealing film. Mix thoroughly by vortexing to obtain a homogenous solution. Centrifuge the collection plate briefly at 5700 x *g* to collect solution from the sealing film. Incubate at 55°C for 10mins until the sample lysate is clear.

Ensure that the deep well collection plate is sealed properly to avoid cross-contamination during shaking.

4. Please refer to Part A for Centrifugation Protocol
Please refer to Part B for Vacuum Protocol

Part A: Centrifugation Protocol

4. Loading to binding plate

Remove the Sealing Film. Transfer the samples carefully into the **96-well DNA Binding Plate** assembled into a **Deep Well Collection Plate.** Do not wet the rims of the wells to avoid aerosol formation during centrifuge. Centrifuge at 5700 x *g* for 5 mins. Discard flow through.

5. Plate washing 1

Add 500µl of **Wash Buffer** into each well carefully. Centrifuge at 5700 x *g* for 5 min. Discard flow through. Repeat the washing step once again.

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

6. Plate drying

Centrifuge the **96-well DNA Binding Plate** at 5700 x *g* for 5 mins or dry the **96-well DNA Binding Plate** at 65°C in an incubator / oven for 10 mins.

It is essential to remove traces of ethanol as it will inhibit downstream applications.

7. DNA elution

Place the **96-well DNA Binding Plate** onto a clean **96-well Storage Plate**. Add 30-50µl of preheated **Elution Buffer,** TE buffer or sterile water to each well and stand for 2mins. Centrifuge at 5700 x *g* for 5 mins. Store DNA at 4°C or -20°C.

Ensure that the Elution Buffer is dispensed directly onto the center of membrane for complete elution. TE buffer can also elute DNA although EDTA may inhibit enzymatic reactions. If water used for eluting DNA, maximum elution efficiency is achieved between pH7.0 and 8.5. Store DNA at -20°C as DNA may degrade in the absence of a buffering agent.

Part B: Vacuum Protocol

4. Loading to binding plate

Place the **96-well DNA Binding Plate** on top of the vacuum manifold. Place a waste tray or a **Deep well Collection Plate** underneath to collect the waste. Remove the **Sealing Film.** Transfer the samples carefully into the **96-well DNA Binding Plate.** Do not wet the rims of the wells to avoid aerosol formation. Apply vacuum at 10-20 inches Hg for 3-5mins until all samples have passed through the **96-well DNA Binding Plate.**

Ensure that the 96-well DNA Binding Plate is fitted properly on the vacuum manifold. If a 2ml 96-well collection plate is used to collect waste, it is necessary to discard the flow through at all times after collection of each buffer flow through, and to blot the top of the plate on paper towels.

5. Plate washing 1

Add 500µl of **Wash Buffer** into each well carefully. Apply vacuum at 10-20 inches Hg for 3-5mins until the buffer has passed through the **96-well DNA Binding Plate.** Repeat the washing step once again.

Ensure that ethanol has been added into Buffer CW1before use (refer to Reconstitution of Solutions).

6. Plate drying

Apply vacuum at 10 inches Hg for additional 10mins, or dry the **96-well DNA Binding Plate** at 65°C in an incubator / oven for 10mins.

It is essential to remove traces of ethanol as it will inhibit downstream applications.

7. DNA elution

Place the **96-well Storage Plate** on top of the waste tray or **Deep Well Collection Plate**, which are both placed inside the vacuum manifold. Place the **96-well DNA Binding Plate** on the vacuum manifold. Add 50-100µl of preheated **Elution Buffer**, TE buffer or sterile water to each well and stand for 2-3mins. Apply vacuum at 10 inches Hg for 2mins. Store DNA at 4°C or -20°C.

Ensure that the Elution Buffer is dispensed directly onto the center of membrane for complete elution. TE buffer can also elute DNA although EDTA may inhibit enzymatic reactions. If water is used for eluting DNA, maximum elution efficiency is achieved between pH7.0 and 8.5. Store DNA at -20°C as DNA may degrade in the absence of a buffering agent.

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
Low DNA yield	*Incomplete sample lysis due to poor resuspension*	*Ensure that samples are completely resuspended in Buffer ACL and* **Proteinase K.** .
	Sample not fresh or not properly stored	*For long term storage of tissues, keep at -70°C.*
	Addition of ethanol was neglected	*Repeat purification again with new samples.*
	Well in binding plate is clogged	*Do not use more than recommended amounts of sample material. If any undigested material remains, spin at maximum speed for 5mins to remove tissue lysate and transfer supernatant in a new* **Deep Well Collection Plate.** *Ensure that* **Wash Buffer** *is applied to the binding plate.*
	Proteinase K *activity is decreased*	*Avoid repeated freeze thaw cycles of* **Proteinase K** *solutions. Ensure that* **Proteinase K** *is stored at -20°C.*
	Wash Solution *reconstituted wrongly.*	*Please refer to "Reconstitution of Solutions". Repeat purification with new samples.*
	DNA Binding Plate *is not dried before addition of* **Elution Buffer**	*Ensure that the* **DNA Binding Plate** *is spin dried by centrifugation at 5700 x g for 10mins or dried at 65°C for 10mins, or apply vacuum for additional 10mins to remove traces of ethanol completely.*
	Elution is not performed properly	*Pre-heat* **Elution Buffer** *at 65-70°C before eluting DNA.*

Problem	Possibility	Suggestions
Low purity ($A_{260/280}$)	**Proteinase K** *activity is decreased*	*Please refer to problem "Low DNA yield".*
	Incomplete protein denaturation	*Use fresh* **Proteinase K** *and extend incubation time until lysate clears.*
DNA degradation smearing	*DNA sheared during purification*	*Add the addition of* **Buffer ACL** *and* **Proteinase K***, avoid vigorous mixing and pipetting. Use cut-off tip if lysate appeared viscous.*
	Sample too old	*DNA already degraded in old sample.*
	Sample frozen and thawed repeated	*Avoid repeated freeze-thaw cycles.*
Poor performance of eluted DNA in downstream applications	*Eluted DNA contains traces of ethanol*	*Ensure that the plate drying step is carried out prior to elution.*
	TE buffer is used to elute DNA. EDTA in TE buffer may inhibit subsequent enzymatic reaction.	*Use* **Elution Buffer** *or water with pH range of 7.0 – 8.5.*