
vivantis
Nucleic Acid Extraction Kit HandBook
96
GF-1
96-WELL GENOMIC DNA
EXTRACTION KIT USER GUIDE
(Version 2.1)
Catalog No.
GF-96-G05: 96 x 5plates
GF-96-G10: 96 x 10plates
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 Genomic DNA Extraction Kits** is designed for rapid and high-throughput purification of genomic DNA from both Gram negative and Gram positive bacteria, 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	5 x 96	10 x 96
Catalog No.	GF-96-G05	GF-96-G10
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
Digestion Buffer	2 x 200ml	4 x 200ml
TE Buffer	150ml	2 x 150ml
Wash Buffer (concentrate)*	3 x 48ml	6 x 48ml
Elution Buffer	30ml	60ml
Proteinase K	2 x 1ml	4 x 1ml
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* Please refer to **Reconstitution of Solutions** and **Storage and Stability** before using this kit.

The **GF-1 96-well Genomic 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%)
RNase A (DNase-free, 20mg/ml)

Reconstitution of Solutions

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

For **GF-96-C05 (5 x 96),**

Add **192ml** of absolute ethanol into the bottle labeled **Wash Buffer** only prior to use.

For **GF-96-C10 (10 X 96),**

Add **192ml** of absolute ethanol into the bottle labeled **Wash Buffer** only prior to use.

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

Storage and Stability

Store 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 **Digestion Buffer** 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

Dogestion Buffer 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 **Digestion Buffer**, incubate at 50°C with occasional mixing until completely dissolved.
- Users are recommended to use a multichannel pipette.

Pre-set incubator / oven to 65°C.
Pre-ser another incubator to 55°C.

1. Sample lysis

a) Transfer 1.6ml overnight bacterial culture per well into **Deep Well Collection Plate**. Seal the plate with a **Sealing Film.** Centrifuge 2-3mins at 2400 x *g*. Discard the supernatant.

b) Add 200µl of **TE Buffer** to each well of the **Deep Well Collection Plate**, and resuspend the cell completely by pipetting.

c) Add 400µl of **Digestion Buffer** to each well, seal the plate with a new **Sealing Film**. Vortex and mix well. Add 3µl of **Proteinase K** into each well, seal and mix well. Incubate at 65°C for 15-30mins until the solution becomes transparent and clear.

Optional: Removal of RNA

If RNA-free DNA is required, add 5µl of RNase A (DNase-free, 50mg/ml) into each sample. Mix and incubate at room temperature for 5mins.

2. Additional of ethanol

Remove the **Sealing Film.** Add 260µl of absolute ethanol into each well. Seal the collection plate properly and firmly with a new **Sealing Film** and mix thoroughly by inverting the plate several times. Centrifuge the collection plate briefly to collect solution from **Sealing Film**.

Ensure that the 96-well collection plate is sealed properly to avoid cross-contamination during inverting.

3. 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. Seal the plate with a new **Sealing Film**. Keep it at room temperature for 2 mins. Centrifuge at 2,400 x *g* for 5 mins. Discard flow through.

5. Plate washing 1

Add 500µl of **Wash Buffer** into each well carefully. Centrifuge at 2,400 x *g* for 5 min. Discard flow through.
Ensure that ethanol has been added into Wash Buffer before use (refer to Reconstitution of Solutions).

6. Plate washing 2

Add 500µl of **Wash Buffer** into each well carefully. Centrifuge at 2,400 x *g* for 5 min. Discard flow through.

7. Plate drying

Centrifuge the **96-well DNA Binding Plate** at 2,400 x *g* for 10mins or dry the **96-well DNA Binding Plate** at 65°C in an incubator / oven for 5mins.
It is essential to remove traces of ethanol as it will inhibit downstream applications.

8. DNA elution

Place the **96-well Storage Plate** on top **Deep Well Collection Plate**, and then place the **96-well DNA Binding Plate** on the top of **96-well Storage Plate**. Add 50µl of **Elution Buffer** into the center part of the membrane of each well and incubate at 55°C for 2mins, centrifuge at 2,400 x *g* for 2mins. Store DNA at 4°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.**
Ensure that ethanol has been added into Wash Buffer before use (refer to Reconstitution of Solutions).

6. Plate washing 2

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.**

7. Plate drying

Apply vacuum at 10-20 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.

8. 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 50µl of preheated **Elution Buffer**, TE buffer or sterile water to each well and incubate at 55°C for 2-3mins. Apply vacuum at 10-20 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 sample are completely resuspended in* **Digestion Buffer** *and* **Proteinase K.** .
	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.**
	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 2,400 x g for 10mins or dried at 65°C for 10mins, or apply vacuum for additional 10mins to remove traces of ethanol completely.*

Problem	Possibility	Suggestions
	Elution is not performed properly	*Incubate column at 55°C for 2-3mins after addition of* **Elution Buffer.** *Ensure that the* **Elution Buffer** *used allow salt buffer or water wit a pH range of 7.0 - 8.5.*
Low purity ($A_{260/280}$)	**Proteinase K** *activity is decreased*	*Please refer to problem "Low DNA yield".*
	Incomplete protein denaturation	*Extend incubation time until lysate clears.*
DNA degration / smearing	*Sample frozen and thawed repeatedly*	*Avoid repeated freeze-thaw cycles.* *DNA already degraded in old sample. Repeat purification with new samples.*
Poor performance of eluted DNA in downstream applications	*Eluted DNA contains traces of ethanol*	*Ensure that the* **DNA Binding Plate** *is spin dried by centrifugation at 2,400 x g for 10mins or dried vacuum for additional 10mins to remove traces of ethanol completely.*
	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.*