vivantis

Nucleic Acid Extraction Kit HandBook

96 GF-1

96-WELL PCR CLEAN-UP KIT USER GUIDE (Version 2.1)

Catalog No.
GF-96-C05: 96 x 5plates
GF-96-C10: 96 x 10plates

Up to 90% recovery of DNA

Purification process takes less than 60 minutes

No organic-based extraction required

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

Introduction

The **GF-1 96-well PCR Clean-Up Kit** is designed for rapid and high-throughput purification of DNA bands ranging from 100bp to 20kb for up to 96 samples simultaneously. The kit contains special buffers to provide the optimum concentration of salt and pH for efficient recovery of DNA. The kit uses a specially-treated glass filter membrane fixed into 96-well format plate to efficiently bind DNA in the presence of high salt. The kit efficiently removes dNTPs, short oligo fragments, mineral oils, enzymes from a PCR reaction product, proteins after restriction enzyme treatment and dephosphorylation, residue of dye residues and ethidium bromide. The kit is also suitable for concentrating DNA, changing of buffers and desalting. The high recovery of pure DNA obtained is ready to use in many routine molecular biology applications such as restriction enzyme digestion, radioactive/fluorescence DNA sequencing, PCR ligation and transformation, probe preparations and other manipulations.

Kit component

Product Catalog No.	5 x 96 GF-96-C05	10 x 96 GF-96-C10
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
Buffer PCR	2 x 75ml	4 x 75ml
Wash Buffer (concentrate)*	4 x 48ml	8 x 48ml
Elution Buffer	30ml	2 x 30ml
Handbook	1	1

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

The **GF-1 96-well PCR Clean-Up 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%)
2ml 96-well collection plates

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 each bottle labeled **Wash Buffer**.

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

Add **192ml** of absolute ethanol into the bottle labeled **Wash Buffer**.

Add **192ml** of absolute ethanol into the other 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 -20°C.
Kit components are guaranteed to be stable for 18 months from the date of manufacture **Buffer PCR** 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 PCR 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 PCR**, incubate at 55°C - 65°C with occasional mixing until 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.

1. Homogenization

Transfer PCR product (up to 50µl) to a clean 2ml 96-well collection plate. Add 250µl of **Buffer PCR** to each well and mix by pipetting.

2. Refer to Part A for Centrifugation Protocol
Refer to Part B for Vacuum Protocol

Part A: Centrifugation Protocol

2. Loading to binding plate

Transfer the samples carefully into the **96-well DNA Binding Plate** assembled into a clean 2ml 96-well collection plate. Do not wet the rims of the wells to avoid aerosol formation during centrifugation. Stand at room temperature for 2 mins. Centrifuge at 5700 x *g* for 5 mins. Discard flow discard.

3. Plate washing

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

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

4. Plate drying

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

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

5. DNA elution

Place the 96-well DNA Binding Plate onto a clean **96-well Storage Plate.** Add 30-50µl of **Elution Buffer,** TE buffer or sterile water to each well and stand for 2 mins. For DNA fragments larger than

8kb, use preheated **Elution Buffer** at 65°C - 70°C for better elution efficiency. Centrifuge at 4500 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 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 the absence of a buffering agent.

Part B: Vacuum Protocol

2. Loading to binding plate

Place the **96-well DNA Binding Plate** on top of the vacuum manifold. Place a waste tray or a 2ml 96-well collection plate underneath to collect the waste. 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.

3. Plate washing

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 by adding 500µl of **Wash Buffer** into each well. 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).

4. 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.

5. DNA elution

Place the **96-well Storage Plate** on top of the waste tray or 2ml 96-well collection plate, inside the vacuum manifold. Place the **96-well DNA Binding Plate** on the vacuum manifold. Add 30-50µl of **Elution Buffer**, TE buffer or sterile water to each well and stand for 2mins. For DNA fragments larger than 8kb, use preheated **Elution Buffer** at 65°C - 70°C for better elution efficiency. 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 recovery of DNA	*Incomplete DNA elution*	*Allow full contact of* **Elution Buffer** *with membrane by dispensing directly onto the center of the filter membrane. Do not elute with less than 30µl of* **Elution Buffer.** .
	Inapprociate **Elution Buffer**	*Ensure that* **Elution Buffer** *used is a low salt buffer or water with a pH range of 7.0-8.5.*
	Wash Buffer *is reconstituted wrongly*	*Please refer to "***Reconstitution of Solutions"***. Repeat purification with new samples.*
	Low elution efficiency	*Pre-heat* **Elution Buffer** *to 65°C-70°C. Add* **Elution Buffer** *to the center of the column.*
	DNA size less than 100bp or greater than 8kb	*Prolong the standing time after adding mixture to the 96-well DNA Binding Plate.*
Poor performance of eluted DNA in downstream applications	*Eluted DNA contains traces of ethanol*	*Ensure that the DNA binding plate is dried by centrifugation or incubation at 65°C for 10 minutes, or apply vacuum for additional 10 minutes 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.*