



vivantis
Nucleic Acid Extraction Kit HandBook

GF-1

PCR CLEAN-UP KIT
USER GUIDE (Version2.2)

Catalog No.

SAMPLE: 5 preps
GF-PC-050: 50 preps
GF-PC-100:100 preps
GF-PC-200:200 preps

High Yield and Purity

Fast and Easy purification in 15 minutes

Reliable and Reproducible

Eluted DNA ready for use in downstream applications

No toxic or organic-based extraction required

Introduction

The **GF-1 PCR Clean Up Kit** is a system designed for rapid clean up of DNA bands ranging from 100bp to 20kb. The **GF-1 PCR Clean Up Kit** contains special buffers to provide the correct salt concentration and pH for efficient recovery (80 - 90%) of DNA. This kit uses a specially treated glass filter membrane fixed into a column to efficiently bind DNA in the presence of high salt. It applies the principle of a mini-column spin technology and is well suited for the removal of excess dNTPs, short oligo fragments, mineral oil, enzymes from a PCR reaction product, proteins after restriction enzyme treatment and dephosphorylation and residual dye and ethidium bromide. This kit also allows for concentration of DNA, changing of buffers and desalting.

Buffer PCR contains a pH indicator for easy determination of the optimal pH for efficient recovery of DNA fragments but at the same time does not interfere with DNA yield and quality as it is thoroughly removed during the subsequent washing steps. 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 components

Product Catalog No.	5 Preps SAMPLE	50 Preps GF-PC-050	100 Preps GF-PC-100	200 Preps GF-PC-200
Components				
GF-1 columns	5	50	100	200
Collection tubes	5	50	100	200
PCR DNA Binding Buffer (Buffer PCR)	5ml	50ml	100ml	2 X 100ml
Wash Buffer (concentrate)*	2.4ml	17ml	34ml	2 X 34ml
Elution Buffer	1.5ml	10ml	20ml	30ml
Handbook	1	1	1	1

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

The **GF-1 PCR Clean Up kit** is available as 50, 100 and 200 purifications per kit.

The reagents and materials provided with the kit are for research purpose only.

Additional Materials to be Supplied by User

Absolute Ethanol (>95%)
3M Sodium Acetate, pH5.2

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-PC-050 (50 preps)**,

Add **40ml** of absolute ethanol into the bottle labeled **Wash Buffer**.

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

Add **80ml** of absolute ethanol into the bottle labeled **Wash Buffer**.

For **GF-PC-200 (200 preps)**,

Add **80ml** of absolute ethanol into the bottle labeled **Wash Buffer**.

Add **80ml** of absolute ethanol into the bottle labeled **Wash Buffer** only prior to use.

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

Storage and Stability

All solutions should be stored at 20°C - 30°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 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.
- **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.
- The amount of **Buffer PCR** provided is sufficient for each purification of 200µl of DNA sample. In the case of inadequate amount of **Buffer PCR**, please make a separate purchase for any additional buffers required.

1. Gel Electrophoresis

Run DNA sample on agarose gel electrophoresis to confirm DNA band if necessary.

2. Homogenization

Determine the volume of sample and adjust to 100µl with sterile distilled water. For DNA samples exceeding 100µl, use directly. Add 5 volumes of **Buffer PCR** to the DNA sample and mix thoroughly by vortexing or inverting tube several times.

If the sample turns to orange/pink/red upon solubilization, add 5µl of 3M sodium acetate pH5.2 and mix thoroughly. The sample should return to yellow.

Ensure that the color of the sample is yellow (pH7 or below) before proceeding to the next step.

3. Loading to column

Transfer the sample into a column (max. 1ml) assembled in a clean collection tube (provided). Centrifuge at 10,000 x g for 1 min. Discard flow through.

Repeat for any remaining sample from step 2.

4. Column washing

Wash the column with 750 μ l **Wash Buffer** and centrifuge at 10,000 x g for 1 min. Discard flow-through.

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

5. Column drying

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

This step has to be carried out to remove all traces of ethanol as residual ethanol can affect the quality of DNA and may subsequently inhibit enzymatic reactions.

6. DNA Elution

Place the column into a clean microcentrifuge tube. Add 30 - 200 μ l of **Elution Buffer**, TE buffer or sterile water directly onto column membrane and stand for 2 min. For DNA fragments larger than 8kb, use preheated elution buffer at 65°C - 70°C for better elution efficiency. Spin at 10,000 x g for 1 min to elute DNA. Store DNA at 4°C or -20°C.

For higher concentration, elute DNA in smaller volume, i.e.: 30 μ l. However, the yield will be slightly reduced. Ensure that the Elution Buffer is dispensed directly onto the center of the membrane for complete elution. TE Buffer can also elute DNA although EDTA may inhibit subsequent 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:

Problems	Possibility	Suggestions
Low recovery of DNA	<i>Incomplete DNA elution</i>	<i>Allow full contact of Elution Buffer with membrane by dispensing directly onto the center of the membrane. Do not elute with less than 30µl of Elution Buffer.</i>
	<i>Inappropriate elution buffer</i>	<i>Ensure that the Elution Buffer used is a low salt buffer or water with a pH range of 7.0 - 8.5.</i>
	<i>The pH in the sample is too high/ pH indicator appears orange/pink/red</i>	<i>Ensure that 3M sodium acetate, pH5.2 is added into the sample prior to loading into column.</i>
	<i>Column not placed at fixed orientation during centrifugation</i>	<i>Place the column which has a triangle mark on the edge, at a fixed position during centrifugation at all times.</i>
Low recovery of DNA larger than 8kb	<i>Low elution efficiency</i>	<i>Preheat Elution Buffer to 65°C - 70°C before eluting DNA.</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>

Gel Electrophoresis

Run DNA sample on agarose gel electrophoresis to confirm DNA band.



Homogenization

Add 5 vol. Buffer PCR.
Mix thoroughly.
Add 5 μ l 3M sodium acetate, pH5.2 (if necessary).

