vivantis

Nucleic Acid Extraction Kit Handbook

GF-1

GEL DNA RECOVERY
USER GUIDE (Version 3.1)

Catalog No.
SAMPLE: 5 Preps
GF-GP-050: 50 preps
GF-GP-100:100 preps
GF-GP-200:200 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 Gel DNA Recovery Kit** is a system designed for rapid purification of DNA bands ranging from 100bp to 10kb from all grades of agarose gel in TAE (Tris-acetate/ EDTA) or TBE (Tris-borate/ EDTA) buffer. The Gel DNA Binding Buffer (**Buffer GB**) is optimized to enhance binding of DNA onto a specially-treated glass filter membrane at pH7.0 or below. High recovery of pure DNA is obtained and ready to use in many routine molecular biology applications such as restriction enzyme digestion, radioactive/ fluorescence DNA sequencing, PCR, ligation, probe preparations and other manipulations.

Kit components

Product Catalog No.	5 Preps SAMPLE	50 Preps GF-GP-050	100 Preps GF-GP-100	200 Preps GF-GP-200
Components				
GF-1 columns	5	50	100	200
Collection tubes	5	50	100	200
Gel DNA Binding Buffer (Buffer GB)	3.2ml	30ml	60ml	2 X 60ml
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 Gel DNA Recovery Kit** is available as 50, 100 and 200 purifications per kit.

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

Additional Materials to be Supplied by User

Absolute Ethanol (>95%)
Isopropanol

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-GP-050 (50 preps)**,

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

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

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

For GF-GP-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 GB 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 GB 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 GB**, incubate at 55°C - 65°C with occasional mixing until precipitate is completely dissolved.
- The amount of **Buffer GB** provided is sufficient for each purification of 0.5g of agarose DNA gel. In the case of inadequate amounts of **Buffer GB,** please make a separate purchase for additional buffer as required.

Pre-set waterbath to 50°C.

1. Gel Electrophoresis

Run DNA sample on agarose gel electrophoresis to separate DNA fragments. Perform ethidium bromide staining for DNA visualization. Cut agarose gel band containing the desired DNA and place it into a pre-weighed microcentrifuge tube.

Ensure that the electrophoresis run is sufficient before performing excision of DNA fragment. Avoid more than 30 sec exposure of UV light onto the DNA.

2. Solubilization of agarose

Determine the nett weight of gel slice and add 1 volume of **Buffer GB** to 1 volume of gel (*A gel slice of mass 0.1g will have a volume of 100µl).* Centrifuge the tube briefly to make sure the gel slice stays at the bottom of the tube. Incubate at 50°C until gel has melted completely. Mix occasionally to ensure complete solubilization.

3. Loading to column

Transfer the sample into a column 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

Add 650µl **Wash Buffer** into the column. 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 - 50µl **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. Centrifuge at 10,000 x *g* for 1 min to elute DNA. Store DNA at 4°C or -20°C.

For higher yield, elute DNA in 50µl and for higher concentration, elute DNA in smaller volume, i.e: 30µl. 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:

Problem	Possibility	Suggestions
Gel slice does not dissolve	*High percentage gel used*	*Extend incubation time with mixing until the gel has completely dissolved.*
	Gel slice is too big	*Minimize gel size by removing extra gel and slice the gel into smaller pieces to enhance solubilization.*
Low recovery of DNA	*Incomplete DNA elution*	*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.*
	TAE or TBE buffer repeatedly used or pH incorrect	*pH of repeatedly used TAE or TBE buffer normally increases. Preferably, use fresh TAE or TBE buffer for each gel electrophoresis run.*
	DNA diffused or released into buffer during electrophoresis, staining and destaining.	*Minimize DNA migration distance during electrophoresis. Do not overlay gel with too much buffer during loading of sample. Minimize staining and destaining time.*

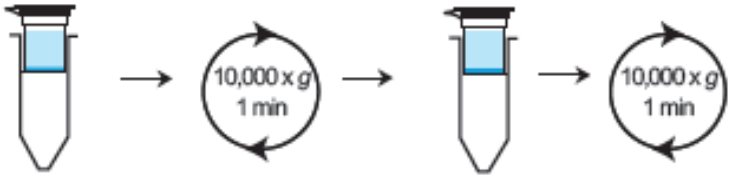
Problem	Possibility	Suggestions
Low recovery of DNA smaller than 400bp	*Elevated temperatures may cause denaturation of DNA into ssDNA*	*Solubilize agarose at 40°C instead of 50°C for an extended period with repeated mixing.*
	Binding efficiency reduced due to small DNA size	*Add 1 gel volume of isopropanol to sample prior to loading onto column.*
Low recovery of DNA larger than 8kb	*Low elution efficiency*	*Pre-heat* **Elution Buffer** *to 65°C -70°C before eluting DNA.*
	Binding efficiency reduced due to large DNA size	*Add 1 gel volume of isopropanol to sample prior to loading onto column.*
No DNA eluted	*Inappropriate elution buffer*	*Ensure that the elution buffer used is a low salt buffer or water with a pH range of 7.0 - 8.5.*
Non-specific DNA fragments appears in eluted DNA	*Migration distance insufficient during electrophoresis*	*Ensure that the electrophoresis run is sufficient to separate bands before performing cut.*
	Scalpel or razor blade used to excise the gel is contaminated with other DNA fragments	*Use a new or clean scalpel or razor blade to excise the gel.*
Poor performance of eluted DNA in downstream applications	*Eluted DNA contains traces of ethanol*	*Ensure that the Column 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 a pH range of 7.0 - 8.5.*

Gel Electrophoresis

Run DNA sample on agarose gel electrophoresis. Cut desired band and determine nett weight of agarose gel.

Solubilization

Add 1 vol. Buffer GB : 1 vol gel. Incubate 50°C until gel has melted completely.



Loading to column

Transfer sample to column

Centrifuge

Discard flow through

Column Washing

Add 650 µl Wash Buffer

Centrifuge

Discard flow through

Column Drying

Elution

Transfer column to a new microcentrifuge tube. Add 30-50µl Elution Buffer. Stand for 2 min.

Centrifuge

Store DNA at 4°C or -20°C

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