



v i v a n t i s

Nucleic Acid Extraction Kit Handbook

GF-1

PLASMID DNA EXTRACTION
USER GUIDE (Version 3.1)

Catalog No.

SAMPLE: 5 preps

GF-PL-050: 50 preps

GF-PL-100: 100 preps

GF-PL-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 Plasmid DNA Extraction Kit** is designed for rapid and efficient purification of high copy and low copy plasmid DNA without the need for precipitation or organic extractions. This it uses a specially-treated glass filter membrane fixed into a column to efficiently bind DNA in the presence of high salt. Combining alkaline lysis-SDS and mini-column spin technology, up to 20µg of plasmid DNA from bacterial cultures can be isolated. Multiple samples can be processed rapidly and with practice, the purification takes less than 30 minutes. Optimized buffers ensure only highly pure plasmid DNA is extracted and is ready to use in many routine molecular biology applications such as restriction enzyme digestion, radioactive/fluorescence DNA sequencing, PCR, ligation, transformation and other manipulations.

	Culture Vol.	Yield	Purity
High Copy Number Plasmid (50-500 copies per cell)	2ml 5ml	2-5µg 10-20µg	1.7-1.9 1.7-1.9
Low Copy Number Plasmid (1-10 copies per cell)	2ml 5ml	1-3µg 5-10µg	1.7-1.9 1.7-1.9

Kit components

Product Catalog No.	5 Preps SAMPLE	50 Preps GF-PL-050	100 Preps GF-PL-100	200 Preps GF-PL-200
Component				
GF-1 columns	5	50	100	200
Collection tubes	5	50	100	200
Solution 1 (S1)*	1.5ml	15ml	30ml	55ml
Solution 2 (S2)* 1	1.5ml	15ml	30ml	55ml
Neutralizing Buffer (Buffer NB)	2.5ml	25ml	50ml	90ml
Wash Buffer (concentrate)*	2.4ml	17ml	34ml	2 X 34ml
RNase A (DNase-free)*	7.5µl	75µl	150µl	275µl
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 Plasmid DNA Extraction 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%)

Reconstitution of Solutions

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

The vial of **RNase A (DNase-Free)** provided is to be added into the bottle labeled **S1**.

For **SAMPLE (5 preps)**,

Add **3.5ml** of absolute ethanol into the bottle labeled **Wash Buffer**.

Add **1ml** of **S1** into the vial of **RNase A** and mix well. Briefly centrifuge and transfer the entire mixture back into the **S1** tube. Mix well.

For **GF-PL-050 (50 preps)**,

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

Add **1ml** of **S1** into the vial of **RNase A** and mix well. Briefly centrifuge and transfer the entire mixture back into the **S1** bottle. Mix well.

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

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

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

Add **1ml** of **S1** into the vial of **RNase A** and mix well. Briefly centrifuge and transfer the entire mixture back into the **S1** bottle. Mix well.

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

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

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

Add **1ml** of **S1** into the vial of **RNase A** and mix well. Briefly centrifuge and transfer the entire mixture back into the **S1** bottle. Mix well.

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

RNase A in **S1** should be stable for 6 months when the solution is stored at 2°C - 8°C.

Storage and Stability

Store **S1** at 2°C - 8°C after the addition of **RNase A**. Store other solutions at 20°C - 30°C.

Ensure that **S2** is closed immediately after use to avoid neutralization with CO₂ in the air. If precipitation occurs in the solution, heat at 37°C. Store at room temperature.

Kit components are guaranteed to be stable for 18 months from the date of manufacture. **Buffer NB** 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 NB 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 and **S1** to be added with **RNase A** before use. Please refer to **Reconstitution of Solutions**.
- If precipitation forms in **Buffer NB**, incubate at 55°C - 65°C with occasional mixing until precipitate is completely dissolved.

1. Preparation of stock culture

Grow 5 - 10ml plasmid-containing bacteria cells in LB medium with appropriate antibiotic(s) overnight (12-16 hours) at 37°C with agitation.

Fresh culture must always be used for extraction.

2. Centrifugation

Pellet 1.5 - 5ml of bacterial culture containing the plasmid by centrifugation at 6,000 x g for 2 min. If 15ml or 50ml centrifuge tube is used to harvest the cells, centrifuge at 6,000 x g for 5 min. Decant the supernatant completely.

Do not centrifuge cells at high speed or for long periods. Cells will become too compact for resuspension.

3. Resuspension of pellet

Add 250µl **S1** to the pellet and resuspend the cells completely by vortexing or pipetting. Transfer the suspension to a clean 1.5ml microcentrifuge tube.

Ensure that cells are completely resuspended. Lysis will not occur if clumps of bacteria remain following an inefficient resuspension procedure. Ensure that RNase A has been added into the S1 buffer before use (refer to Reconstitution of Solutions).

4. Alkaline lysis

Add 250µl of **S2** and **gently mix** by inverting tube several times (4-6 times) to obtain a clear lysate. Incubate on ice or at room temperature for **NOT** longer than 5 min.

Do NOT vortex! Vortexing shears the genomic DNA and leads to contamination with chromosomal DNA.

S2 should be immediately capped tightly after used.

Incubation on ice may reduce non-supercoiled plasmid contamination in some bacteria strains.

Precipitation of SDS and cell debris in the following Neutralization step will be slightly more effective in the cold.

5. Neutralization

To neutralize the lysate, add 400µl of **Buffer NB** and **gently mix** by inverting the tube several times (6-10 times) until a white precipitate forms. Centrifuge at 14,000 - 16,000 x g for 10 min.

Do NOT vortex upon addition of Buffer NB! Vortexing shears the genomic DNA and leads to contamination with chromosomal DNA.

After centrifugation, the compact white precipitate should be spun down and separated from the supernatant.

6. Loading to column

Transfer 650µl of **supernatant** into a column assembled in a clean collection tube (provided).

Centrifuge at 10,000 x g for 1 min. Discard flow through.

Repeat for the remaining sample from step 5.

Be careful not to transfer any white precipitate into the column.

7. Column washing

Wash the column with 650µl **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).

8. 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

9. DNA elution

Place the column into a clean microcentrifuge tube. Add 50 - 100µl of **Elution Buffer**, TE buffer or sterile water directly onto column membrane and stand for 1 min. Centrifuge at 10,000 x g for 1 min to elute DNA.

Store DNA at 4°C or -20°C.

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
Low DNA yield	<i>Cell lysis incomplete / Lysate did not clear after addition of S2</i>	<i>Do not exceed the recommended culture volume of 5ml.</i> <i>Use fresh S2 by preparing as follows: 0.2N NaOH, 1% SDS.</i>
	<i>Poor resuspension of cells</i>	<i>Ensure that cells are completely resuspended after the addition of S1. No cell clumps should be visible.</i>
	<i>Low copy-number plasmid</i>	<i>Increase culture volume or grow culture in enriched medium such as Terrific Broth to increase the yield.</i>
	<i>Bacteria culture overgrown or not fresh</i>	<i>Do not culture bacteria longer than 20 hours at 37°C as this may lower the plasmid yield. Media should contain antibiotic at an appropriate concentration.</i>
	<i>Elution is not performed properly</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>
Column clogged	<i>Transfer of precipitate from sample prior to loading into column</i>	<i>Ensure that white precipitate is not transferred over during loading of column to prevent clogging up of the membrane.</i>

Problem	Possibility	Suggestions
High molecular weight DNA contamination.	<i>Vigorous mixing of lysate upon addition of S2 or Buffer NB</i>	<i>Do not vortex or mix vigorously after addition of S2 or Buffer NB. Simply mix by gently inverting the tube a few times.</i>
	<i>Incubation longer than 5 min after addition of S2</i>	<i>Do not incubate longer than 5 min.</i>
Additional plasmid formation	<i>Irreversible denaturation during cell lysis</i>	<i>Do not carry out incubation longer than 5 min after the addition of S2.</i>
	<i>Nicked circular plasmids due to the presence of nucleases</i>	<i>Carry out purification without delay at least until the washing step where nucleases will be removed.</i>
		<i>Incubation on ice after addition of S2 reduces nuclease activity.</i>
RNA Contamination	<i>RNA digestion was insufficient</i>	<i>Ensure that RNase A has been added into S1 or add a new preparation of RNase A into S1 to a final concentration of 100µg/ml.</i>
Poor performance of eluted DNA in downstream applications	<i>Eluted DNA contains traces of ethanol</i>	<i>Ensure that the Column drying step carried out prior to elution.</i>
	<i>TE buffer is used to elute DNA. EDTA in TE buffer may inhibit subsequent enzymatic reaction</i>	<i>Use Elution Buffer or water with a pH range of 7.0 - 8.5.</i>

