

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	2ml	2-5µg	1.7-1.9
(50-500 copies per cell)	5ml	10-20µg	1.7-1.9
Low Copy Number Plasmid	2ml	1-3µg	1.7-1.9
(1-10 copies per cell)	5ml	5-10µg	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	7.5µl	75µ1	150µl	275µ1
(DNase-free)*	•		•	•
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 CO_2 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 $250\mu l$ 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 400μ l of **Buffer NB** and **gently mix** by inverting the tube several times (6-10 times) until a white precipitate forms. Centrifuge at $14,000 - 16,000 \times g$ 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 $50 - 100\mu l$ of **Elution Buffer**, TE buffer or sterile water directly onto column membrane and stand for 1 min. Centrifuge at $10,000 \times g$ for 1 min to elute DNA. Store DNA at 4° C or -20° C.

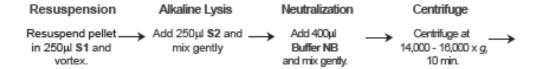
Ensure that the elution buffer is dispensed directly onto the center of the membrane for complete elution. TE Buffer can also elute DNA although EDTA may inhibit subsequent enzymatic reactions. If water is used for eluting DNA, maximum elution efficiency is achieved between pH7.0 and 8.5. Store DNA at -20°C as DNA may degrade in the absence of a buffering agent.

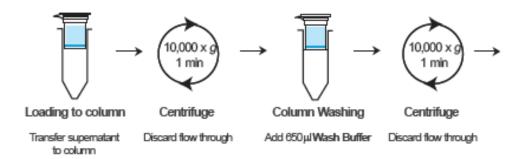
Troubleshooting

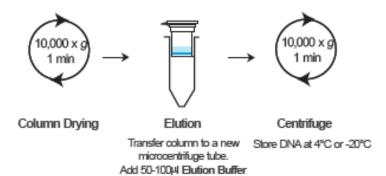
Please note that by not adhering to the recommended protocols, unsatisfactory results related to yield and quality of DNA may occur. If problems arise, please refer to the following:

Problem	Possibility	Suggestions
Low DNA yield	Cell lysis incomplete / Lysate did not clear after addition of S2	Do not exceed the recommended culture volume of 5ml.
		Use fresh S2 by preparing as follows: 0.2N NaOH, 1% SDS.
	Poor resuspension of cells	Ensure that cells are completely resuspended after the addition of S1 . No cell clumps should be visible.
	Low copy-number plasmid	Increase culture volume or grow culture in enriched medium such as Terrific Broth to increase the yield.
	Bacteria culture overgrown or not fresh	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.
	Elution is not performed properly	Ensure that the elution buffer used is a low salt buffer or water with a pH range of 7.0 - 8.5.
Column clogged	Transfer of precipitate from sample prior to loading into column	Ensure that white precipitate is not transferred over during loading of column to prevent clogging up of the membrane.

Problem	Possibility	Suggestions
High molecular weight DNA contamination.	Vigorous mixing of lysate upon addition of S2 or Buffer NB	Do not vortex or mix vigorously after addition of S2 or Buffer NB . Simply mix by gently inverting the tube a few times.
	Incubation longer than 5 min after addition of S2	Do not incubate longer than 5 min.
Additional plasmid formation	Irreversible denaturation during cell lysis	Do not carry out incubation longer than 5 min after the addition of S2 .
	Nicked circular plasmids due to the presence of nucleases	Carry out purification without delay at least until the washing step where nucleases will be removed.
		Incubation on ice after addition of S2 reduces nuclease activity.
RNA Contamination	RNA digestion was insufficient	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.
Poor performance of eluted DNA in downstream applications	Eluted DNA contains traces of ethanol	Ensure that the Column drying step 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.







or water. Stand for 1 min.