vivantis

Nucleic Acid Extraction Kit HandBook

96 GF-1

96-WELL PLASMID DNA EXTRACTION KIT USER GUIDE (Version 2.1)

Catalog No.
GF-96-P05: 96 x 5 plates
GF-96-P10: 96 x 10 plates

Yields up to 10μg of plasmid 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 Plasmid DNA Extraction Kit** is designed for rapid and high-throughput purification of high copy number and low copy number plasmid DNA for up to 96 samples simultaneously without the need for precipitation or organic extractions. This kit uses specially-treated glass filter membrane fixed into a 96-well format plate to efficiently bind DNA in the presence of high salt. 5-10µg of high copy number plasmid and 0.5-5µg of low copy number plasmid can be isolated from bacterial cultures. Optimized buffers ensure only highly pure plasmid DNA is extracted while other contaminants are removed during subsequent washing steps. The purification can be completed in 60 mins and the eluted plasmid is ready to use in many routine molecular biology applications such as restriction enzyme digestion, radioactive / fluorescence DNA sequencing, PCR, transformation and other manipulations.

Kit component

Product	5 x 96	10 x 96
Catalog No.	GF-96-P05	GF-96-P10
Components		
GF-1 96-well Filter Plate	5	10
GF-1 96-well DNA Binding Plate	5	10
Deep Well Collection Plate	10	20
96-well Storage Plate	5	10
Sealing Film	25	50
Buffer PD1*	60ml	2 x 60ml
Buffer PD2*	120ml	2 x 120ml
Buffer PD3	210ml	2 x 210ml
Wash Buffer (concentrate)*	4 x 48ml	8 x 48ml
Elution Buffer	30ml	2 x 30ml
RNase A*	1.2ml	2 x 1.2ml
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* Please refer to **Reconstitution of Solutions** and **Storage and Stability** before using this kit.

The **GF-1 96-well Plasmid DNA Extraction 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%)

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 **Buffer PD1**.

For **GF-96-P05 (5 x 96),**

Add **192ml** of absolute ethanol into each bottle labeled **Wash Buffer**.
Add 0.5ml of **Buffer PD1** into the vial of **RNase A** and mix well. Briefly centrifuge and transfer the entire mixture back into the **Buffer PD1** bottle. Mix well.

For **GF-96-P10 (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.
Add 0.5ml of **Buffer PD1** into the vial of **RNase A** and mix well. Briefly centrifuge and transfer the entire mixture back into the **Buffer PD1** bottle. Mix well.
Add 0.5ml of **Buffer PD1** into the other vial of **RNase A** only prior to use and mix well. Briefly centrifuge and transfer the entire mixture back into the **Buffer PD1** bottle. Mix well.

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

Storage and Stability

Store **Buffer PD1** at 2°C - 8°C after the addition of **RNase A. RNase A** in **Buffer PD1** should be stable for 18 months when the solution is stored at 2°C - 8°C.
Store solutions at 20°C – 30°C.
Ensure that **Buffer PD2** 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 12 months from the date of manufacture.
Buffer PD3 may exhibit salt precipitation due to cold temperature. If this occurs, simply warm the bottle at 55°C - 65°C with occasional mixing until the precipitation is completely dissolved.

Chemical Hazard

Buffer PD1 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 **Buffer PD1** to be added with **RNase A** before use. Please refer to **Reconstitution of Solutions**.
- If precipitation forms in **Buffer PD3**, 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. Harvest bacterial cells

Transfer 1.5ml of bacterial culture into a 96-well collection plate without wetting the rims of the walls. Centrifuge at 5,700 x *g* for 5-10mins. Discard the medium and blot the top of the collection plate on paper towels to remove any remaining media.

2. Resuspension of bacterial pellet

Add 100µl **Buffer PD1** to each well of the plate and resuspend the cell pellet by pipetting or shaking.

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 Buffer PD1 before use (refer to Reconstitution of Solutions).

3. Alkaline lysis

Add 200µl **Buffer PD2** to the suspension. Seal the collection plate properly and firmly with **Sealing Film** and gently invert the collection plate 4-6 times. Centrifuge the collection plate briefly at 2,400 x *g* to collect solution from the **Sealing Film.** Stand at room temperature for 3-5mins until the suspension appears clear.

Ensure that the 96-well collection plate is sealed properly to avoid cross-contamination during inversion. Do not shake the plate vigorously as this will shear the genomic DNA and lead to contamination with chromosomal DNA. Do not incubate for more than 5 minutes. Additional incubation can result in increased levels of open circular Plasmid DNA.

4. Neutralization

Remove the **Sealing Film**. Add 350µl **Buffer PD3** to each well. Seal the collection plate properly and firmly with a new **Sealing Film** and invert the plate 6-10 times. Incubate for 3 minutes. Centrifuge the 96-well Collection Plate at 4,500 x *g* for 10mins. Remove the Sealing Film.

Ensure that the 96-well collection plate is sealed properly to avoid cross-contamination during inversion.

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

Part A: Centrifugation Protocol

5. Loading to Filter Plate

Place a new **Deep Well Collection Plate** in the base, and put a clean 96-well Filter Plate on top. Pipette the clear lysates carefully into **96-well Filter Plate**. Centrifuge at 5,700 x *g* for 5mins. Collect lysate in the Deep Well Collection Plate.

6. Loading to Binding Plate

Put a clean **96-well DNA Binding Plate** on top of a new **Deep well Collection Plate**. Transfer the lysate from step 5 carefully into **96-well DNA Binding Plate** assembled into a clean deep well collection plate. Centrifuge at 5,700 x *g* for 5mins. Discard flow through.

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

7. Plate washing

Add 500µl **Wash Buffer** into each well carefully. Centrifuge at 5,700 x *g* for 5mins. Repeat washing by addition of 500µl **Wash Buffer** into each well and centrifuge at 5,700 x *g* for 5mins. Discard flow through.

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

8. Plate drying

Centrifuge the **96-well DNA Binding Plate** at 5700 x *g* for 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.

9. DNA elution

Place the **96-well DNA Binding Plate** onto a clean **96-well Storage Plate.** Add 50-100µl of **Elution Buffer,** TE buffer or sterile water to each well and stand for 2mins. Stand at room temperature for 2mins. Centrifuge at 4,500 x *g* for 2mins to elute DNA. 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

5. Loading to Filter Plate

Place a new **96-well Filter Plate** on top of vacuum manifold. Place a new deep well collection plate underneath to collect the lysate.
Pipette the lysates carefully into the **96-well Filter Plate**. Apply vacuum at 10 inches Hg for 3-5mins until all samples have pass through. Collect lysate in the deep well collection plate.

6. Loading to binding plate

Place the **96-well DNA Binding Plate** on top of the vacuum manifold. Place a waste tray or a deep well collection plate underneath to collect the waste. Transfer the cleared lysate from step 5 carefully into the **96-well DNA Binding Plate**. Do not wet the rims of the wells to avoid aerosol formation. Apply vacuum at 10 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.

7. Plate washing

Add 500µl of **Wash Buffer** into each well carefully. Apply vacuum at 10 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 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).

8. Plate drying

Apply vacuum at 10 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.

9. DNA elution

Place the **96-well Storage Plate** on top of the waste tray or deep well collection plate, inside the vacuum manifold. Place the **96-well DNA Binding Plate** on the vacuum manifold. Add 50-100µl of **Elution Buffer**, TE buffer or sterile water to each well and stand for 2mins. Apply vacuum at 10 inches Hg for 3-5mins. 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 DNA yield	*Poor resuspension of cells*	*Ensure that cells are completely resuspended after addition of* **Buffer PD1**. *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 cells overgrown or not fresh	*Do not culture bacteria for more than 20 hours at 37°C as this may lower the plasmid yield. Media should contain antibiotic at an appropriate concentration.*
	Buffer PD2 *precipitated*	*Heat at 37°C until precipitate is dissolved.*
	Well in the **DNA Binding Plate** *is clogged*	*Ensure that white precipitate is not transferred over during loading to column to prevent clogging up of the filter membrane.*
High molecular weight DNA contamination	*Vigorous mixing of lysate upon addition of* **Buffer PD2** *and* **Buffer PD3**	*Do not mix vigorously after addition of* **Buffer PD2** *and* **Buffer PD3**. *Simply mix by gently inverting the plate for a few times.*
	Incubation longer than 5mins after addition of **Buffer PD2**	*Do not incubate more than 5mins.*

Problem	Possibility	Suggestions
Additional plasmid form	*Irreversible denaturation during cell lysis*	*Do not carry out incubation longer than 5mins after addition of* **Buffer PD2.** *Repeat purification with new samples.*
	Nicked circular plasmids due to the presence of nucleases	*Incubation on ice after addition of* **Buffer PD2** *reduces nucleases activity.*
RNA contamination	*RNA digestion is insufficient*	*Ensure that* **RNase A** *has been added into* **Buffer PD1** *or add a new preparation of* **RNase A** *into* **Buffer PD1** *to a final concentration of 100µg/ml.*
Poor performance of eluted DNA in downstream applications	*Eluted DNA contains traces ethanol*	*Ensure that the* **DNA Binding Plate** *is dried by centrifugation or incubation at 65°C for 10mins, or apply vacuum for additional 10mins 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 a pH range of 7.0 – 8.5*