



vivantis

Nucleic Acid Extraction Kit HandBook

GF-1

**BACTERIAL DNA EXTRACTION
USER GUIDE (Version 3.1)**

Catalog No.

SAMPLE: 5 preps

GF-BA-050: 50 preps

GF-BA-100:100 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 Bacterial DNA Extraction Kit** is designed for rapid and efficient purification up to 20µg of a high molecular weight genomic DNA from either Gram-negative or Gram –positive bacteria. This kit uses a specially-treated glass filter membrane fixed into a column to efficiently bind DNA in the presence of high salt. This kit applies the principle of a mini-column spin technology and the use of optimized buffers to ensure that only DNA is isolated while cellular proteins, metabolites, salts and other low molecular weight impurities are removed during the subsequent washing steps. High-purity genomic DNA is eluted in water or low salt buffers and has a $A_{260/280}$ ratio between 1.7 and 1.9 making it ready-to-use in many routine molecular biology applications such as restriction enzyme digestion, Southern blotting, PCR, DNA fingerprinting and other manipulations.

Kit component

Product Catalog No.	5 Preps SAMPLE	50 Preps GF-BA-050	100 Preps GF-BA-100
Components			
GF-1 columns	5	50	100
Collection tubes	5	50	100
Resuspension Buffer 1 (Buffer R1)	1ml	8ml	15ml
Resuspension Buffer 2 (Buffer R2)	1.5ml	12ml	24ml
Bacterial Genomic Binding Buffer (Buffer BG)	4ml	28ml	56ml
Wash Buffer	2.4ml	17ml	34ml
Elution Buffer	1.5ml	10ml	20ml
Proteinase K*	0.11ml	1.05ml	2 x 1.05ml
Handbook	1	1	1

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

The **GF-1 Bacterial DNA Extraction Kit** is available as 50 and 100 purifications per kit.
The reagents and materials provided with the kit are for research purposes only

Note: The **GF-1 Bacterial DNA Extraction Kit** is optimized to isolate up to 20µg of DNA on recommended cell culture volume. Bacteria cultures vary in the number of cells depending on the strain, growth conditions and viability of the cells. When processing samples, do not use more than the recommended starting volume as it will lead to excessive number of cells and overloading of the column. This would result on reduced yield and purity. The recommended cell culture volume is between 1 to 3ml for both Gram-negative and Gram-positive bacteria to ensure yield and purity is obtained.

Additional Materials to be Supplied by User

Absolute Ethanol (>95%)

Lysozyme (50mg/ml)

RNase A (DNase-free) (20mg/ml)

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-BA-050 (50 preps)**,

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

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

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

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

Storage and Stability

Store solutions at 20°C – 30°C.

Proteinase K is stable for up to 1 year after delivery when stored at room temperature or 4°C. To prolong the lifetime of Proteinase K, storage at -20°C is recommended.

Kit components are guaranteed to be stable for 18 months from the date of manufacture **Buffer BG** 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 BG 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 BG**, incubate at 55°C - 65°C with occasional mixing until completely dissolved.

Pre-set waterbath to 37°C and the second waterbath to 65°C.

Pre-heat **Elution Buffer** to 65°C (optional).

1. Centrifugation

Pellet 1 - 3ml of bacteria culture grown overnight or culture grown to log phase by centrifugation at 6,000 x g for 2 min at room temperature. Decant the supernatant completely.

Thorough removal of supernatant is essential as residual culture media may affect both yield and purity.

2. Resuspension of pellet

Add 100µl **Buffer R1** to the pellet and resuspend the cells completely by pipetting up and down.

Ensure complete cell resuspension. Lysis will not occur if clumps of bacteria remain following an inefficient resuspension procedure.

3. Lysozyme treatment

For Gram-negative bacteria strains, add 10µl lysozyme (50mg/ml) into the cell suspension.

For Gram-positive bacteria strains, add 20µl lysozyme (50mg/ml) into the cell suspension.

Mix thoroughly and incubate at 37°C for 20 min.

Some bacterial strains may require longer incubation time in lysozyme.

4. Centrifugation

Pellet digested cells by centrifugation at 10,000 x g for 3 min. Decant the supernatant completely.

5. Protein denaturation

Resuspend pellet in 180 µl of **Buffer R2** and add 20 µl of **Proteinase K**. Mix thoroughly. Incubate at 65°C for 20 min in a shaking waterbath or with occasional mixing every 5 min.

Lysate should be clear at the end of incubation or else extend the incubation time to 30 min.

Optional: Removal of RNA

If RNA-free DNA is required, add 20 µl of RNase A (DNase-free, 20mg/ml). Mix and incubate at 37°C for 5 min.

Residual RNA fragments will be removed during column washing.

6. Homogenization

Add 2 volumes (~400 µl without RNase A treatment, ~440 µl with RNase A treatment) of **Buffer BG** and mix thoroughly by inverting tube several times until a homogeneous solution is obtained. Incubate for 10 min at 65°C.

7. Addition of Ethanol

Add 200 µl of absolute ethanol. Mix immediately and thoroughly.

Mix immediately to prevent uneven precipitation of nucleic acid due to high local ethanol concentrations.

8. Loading to column

Transfer the sample (max.650µl) into a column assembled in a clean collection tube (provided). Centrifuge at 10,000 x g for 1 min. Discard flow through.

If column clogs, add 200µl Buffer BG into column and centrifuged as above.

9. Column washing

Wash the column with 650 µl of **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).

10. 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.

11. DNA elution

Place the column into a clean microcentrifuge tube. Add 50 - 100 µl of preheated **Elution Buffer**, TE buffer or sterile water directly onto column membrane and stand for 2 min. Centrifuge at 10,000x 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>Incomplete cell resuspension</i>	<i>Ensure that cells are completely resuspended in Buffer R1 and Buffer R2 before incubation in lysozyme and Proteinase K.</i>
	<i>Low elution efficiency</i>	<i>Pre-heat Elution Buffer to 65°C -70°C before eluting DNA.</i>
	<i>Column clogged</i>	<i>Refer to Problems under 'Column clogged'.</i>
Low purity	<i>Incomplete protein denaturation</i>	<i>Ensure that cells are completely resuspended in Buffer R1 and Buffer R2 before incubation in lysozyme and Proteinase K. Extend incubation time until lysate clears.</i>
	<i>RNA contamination</i>	<i>Add RNase A to the sample as indicated in the protocol. Ensure that RNase used has not been repeatedly frozen and thawed. If necessary prepare a fresh stock.</i>
Difficult to resuspend cell pellet in Buffer R1 or R2	<i>Centrifugation at high speed and long periods</i>	<i>Ensure that the cell culture is centrifuged at the recommended speed and time.</i>
		<i>Using pipette tips, pipette the lysate up and down until completely homogenized.</i>

Problem	Possibility	Suggestions
No DNA eluted	<i>Inappropriate elution buffer</i>	Ensure that the Elution Buffer used is a low salt buffer or water with a pH range of 7.0-8.5.
Lysate not clear after incubation with Buffer R2	<i>Lysozyme activity may decrease with time</i>	Ensure that lysozyme is fresh and has not been repeatedly frozen and thawed.
Column clogged	<i>Lysate is not clear due to insufficient digestion or lysis</i>	Ensure that the lysate is clear prior to sample loading onto the column. If necessary, extend incubation time in lysozyme and Proteinase K .
	<i>Overloading of column / starting culture volume too high</i>	Use the recommended culture volume between 1 - 3ml.
DNA degradation / smearing	<i>DNA sheared during purification</i>	After the addition of Proteinase K , avoid vigorous mixing and pipetting. Mix gently by inverting tube.
	<i>Nuclease contamination</i>	Use sterilized glassware, plasticware and wear gloves. Ensure that cells are completely resuspended in Buffer R2 .
Poor performance of eluted DNA in downstream applications	<i>Eluted DNA contains traces of ethanol</i>	Ensure that the Column drying step is carried out prior to elution.

