# EasyPrep™ Bacterial Genomic DNA Miniprep Manual

Catalog#: GD04-01, GD04-02

Bioland

**For research use only**

**(March 2024)**

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*Limited Use and Warranty*

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## Introduction

The EasyPrep™ Bacterial gDNA kit provides a rapid and reliable method for isolating high-quality total cellular DNA from a wide variety of bacterial species. Bacterial cells are grown to log-phase and harvested. The bacterial cell wall is digested by lysozyme and Proteinase K. Following lysis, binding conditions are adjusted and the sample is applied to a DNA column.

Key to this kit is our proprietary DNA binding system that allows the high efficient binding of DNA to the membrane while proteins and other contaminates are removed under optimal conditions. Nucleic acids are easily eluted with Elution Buffer. Purified DNA is suitable for PCR, restriction digestion, and hybridization. Each DNA column can bind to ~100 µg genomic DNA and process up to 1 x $10^9$ bacterial cells.

## Kit Contents

| Product | GD04-01 | GD04-02 | |
|---|---|---|---|
| DNA Mini Columns | 50 | 250 | RT |
| 2 mL Collection Tubes | 100 | 500 | RT |
| Buffer BL | 6 ml | 30 ml | RT |
| Buffer GD | 60 ml | 270 ml | RT |
| DNA Wash Buffer (5x) | 12 ml | 54 ml | RT |
| Elution Buffer | 15 ml | 75 ml | RT |
| Column Buffer | 6 ml | 30 ml | RT |
| Lysozyme (50mg/ml) | 50 mg | 250 mg | -20°C |
| Proteinase K (20mg/ml) | 12 mg | 60 mg | -20°C |
| RNase A (20mg/ml) | 120 µl | 600 ul | 4°C |
| Manual | 1 | 1 | |

** Buffer GD, KB contains chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the preparation waste.*



## Storage and Stability

The shelf life for this kit is 12 months from the date of purchase.

- Once reconstituted, proteinase K and lysozyme must be stored at -20°C.
- Store RNase A at 4°C.
- Other components can be stored at 22°C-25°C.

## Before Starting

Please go over the handbook and get familiar with the procedure.

- Prepare a stock solution of Proteinase K (provided) as follows and aliquot into adequate portions. Store aliquots at -20°C. Bring Proteinase K solution to room temperature before use.

| | |
|---|---|
| GD04-01 | Dissolve with 0.6 mL of Elution Buffer |
| GD04-02 | Dissolve with 3 mL of Elution Buffer |

- Prepare a lysozyme stock solution at 50 mg/mL and aliquot into adequate portions. Store each aliquot at -20°C.

| | |
|---|---|
| GD04-01 | Dissolve with 1 mL of Elution Buffer |
| GD04-02 | Dissolve with 5 mL of Elution Buffer |

- Add 100% ethanol to DNA Wash Buffer and store at RT

| | |
|---|---|
| GD04-01 | Add 48 mL absolute ethanol (96%-100%) |
| GD04-02 | Add 216 mL absolute ethanol (96%-100%) |

- Carry out all of centrifugation step at room temperature. Warm up Elution Buffer provided to 65°C.

## Materials to Be Provided by User

- Tabletop microcentrifuge and nuclease-free 1.5 mL tubes
- Waterbath set to 37°C
- Shaking waterbath set to 55°C
- Incubator or waterbath set to 65°C
- Absolute ethanol (96%-100%)

## Centrifuge Protocol

This method is designed for isolating gDNA from 1-3 mL log phase bacteria culture grown in LB medium. Overnight culture can be used in many cases.

1. **Pellet 1-3 mL culture by centrifugation at 12,000 x g for 2 min at room temperature.**
2. **Discard medium completely and resuspend the pellet in 180 µL Elution Buffer. Add 18 µL 50 mg/mL lysozyme solution and 2 µL RNase A, incubate at 37°C for 15-30 min.** *Note: Complete digestion of the cell wall is essential for efficient lysis. Longer incubation time may yield more genomic DNA.*
3. **Centrifuge the cells at 5,000 x g for 5 min at room temperature. Discard supernatant and leave 10µl residual liquid in the tube. Resuspend the cell pellet by vortexing.**
4. **Add 100 µL Buffer BL and 10 µL Proteinase K and vortex for 10 seconds. Spin down briefly. Incubate the mixture at 55°C in a shaking waterbath for 30 min. If no shaking waterbath is available, briefly vortex the samples every 2-3 min.**
5. **Add 500 µl Buffer GD, vortex to resuspend the cell pellet. Homogenize the lysate by passing it through a 20-gauge needle fitted to a DNase free syringe for at least 5 times.**
6. **(Optional) Column equilibration: add 100 µl Column Buffer to the gDNA binding column, incubate for 2 min, centrifuge at 11,000 rpm for 30-60s.** *This ensures the column having optimal binding capacity. Especially when the kit is stored for a while.*
7. **Transfer the homogenized lysate to a gDNA binding column. Centrifuge at 11,000 rpm for 30-60s. Discard the flowthrough.**
8. **Transfer the column into a new 2 mL tube and add 500 µL Buffer GD. Centrifuge at 10,000 x g for 1 min. Discard flow-through and reuse the collection tube.**
9. **Place the column into the same collection tube and wash by adding 500 µL DNA Wash Buffer diluted with ethanol. Centrifuge at 10,000 x g for 1 min.**
10. **Add 500 µL DNA Wash Buffer to column. Centrifuge at 10,000 x g for 1 min. Discard flow-through and put the column back into the collection tube.**
11. **Centrifuge the column at 10,000 x g for 2 min to dry the column.** *Note: Residue ethanol will be removed more efficiently with the column lid open.*
12. **Place the column into a nuclease-free 1.5 mL microfuge tube and add 100 µL pre-warmed (65°C) Elution Buffer onto DNA Mini column membrane. Allow columns to incubate at 65°C for 5 min.** *Note: Incubating the DNA column at 65°C for 5 min prior to centrifugation will give a modest increase in DNA yield.* **Centrifuge at 10,000 x g for 1 min to elute the DNA.**
13. **(Optional) Repeat the elution with a second 100 µL Elution Buffer.**

**Note:** Each 100 µL elution typically yields 60-70% of the DNA bound to the column. Thus two elutions generally give ~90%. However, increasing elution volume reduces the concentration of the final product. To obtain DNA with high concentrations, elution can be carried out using 50 µL Elution Buffer. Volumes lower than 50µL greatly reduce yields. In some instances yields may be increased by incubating the column at 70°C upon addition of Elution Buffer.

The purified DNA is stable in elution buffer if stored at -20°C or below.

## Trouble Shooting

| | | |
|---|---|---|
| **Column clogged** | Over amount of samples | Do not use greater than 3 mL culture at $OD_{600}$ =1.0 or 1 x $10^9$ bacterial cells per spin column. |
| | Lysis not complete | Add the correct volume of Buffer BL and incubate at 55°C to obtain complete lysis. It may be necessary to extend incubation time to 30 min |
| | Cell remains | Add more lysozyme or increase the incubation time. It may be necessary to increase incubation to 30 min |
| **Low DNA Yield** | Clogged column | See above |
| | Poor elution | Repeat elution or increase elution volume (see note on page 6). Incubation of column at 65°C for 5 min after addition of Elution Buffer may increase yields. |
| | Improper washing | DNA Wash Buffer must be diluted with absolute (96%-100%) ethanol |
| **Low $A_{260}/A_{280}$ Ratio** | Extended centrifugation during elution step | Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation. It will not interfere with PCR or restriction digests. |
| | incomplete mixing with Buffer GD | Repeat the procedure, this time making sure to vortex the sample with Buffer BL immediately and completely. |
| | Insufficient incubation | Increase incubation time with Buffer BL. Ensure that no visible cell clumps remain |
| | Trace protein contamination | Following step 7, wash column with 300 µL Buffer KB before proceeding to step 8. |


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