For general laboratory use

Gene Transfer Pioneers
(GTP)

Genomic DNA Extraction Kit from Gram Positive Bacteria

Kit for 25 purifications

Catalogue Number: DM05025

Store the kit at +15 to +25°C.

Introduction

The **Genomic DNA Extraction Kit from Gram Positive Bacteria** provides a quick and easy method for purifying total DNA (including genomic DNA) from various gram positive bacteria. Homogenized samples are treated with RNase A. In the presence of the binding buffer, coupled with chaotropic salt, genomic DNA in the lysate binds to the glass fiber matrix of the spin column. Contaminants are removed using a Wash Buffer (containing ethanol) and the purified genomic DNA is eluted by a low salt Elution Buffer, TE or water. DNA phenol extraction or alcohol precipitation is not required and can be completed in less than 60 minutes. The purified genomic DNA is ready for use in PCR, Real-time PCR, Southern Blotting and RFLP.

Quality Control

The quality of the **Genomic DNA Extraction Kit from Gram Positive Bacteria** is tested on a lot-to-lot basis according to Gene Transfer Pioneers quality management system. Genomic DNA is isolated from 50 mg bacteria. More than 10 µg of genomic DNA is quantified with a spectrophotometer and analyzed by electrophoresis.

Kit Contents

All solutions are clear. If any solution contains a precipitate, do not use it. Instead, warm the solution at room temperature or in a 37°C water bath to dissolve the precipitate.

Vial	Label	Contents
1. White Cap	DM1 Buffer	10 ml
2. White microtube	RNase A	55 µl
3. White microtube	Lysozyme	20 µl
4. White microtube	Proteinase K	550 µl
5. Brown Cap	DM2 Buffer	10 ml
6. Blue Cap	Wash Buffer	3 ml; add 12 ml ethanol
7. Black Cap	Elution Buffer	10 ml
8. Colorless Bag	DNA Spin Column	25 Columns

Storage and Stability

Store the **Genomic DNA Extraction Kit from Gram Positive Bacteria** components at +15 to +25°C. Kit components are guaranteed to be stable date printed on the label.

Application

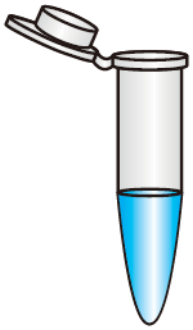
Isolation of up to 20 µg purified DNA from yeast, which may be used directly in downstream applications such as restriction enzyme digestion, PCR, sequencing, *in vitro* transcription or labeling reactions.

Before You Begin: Preparation of Working Solutions

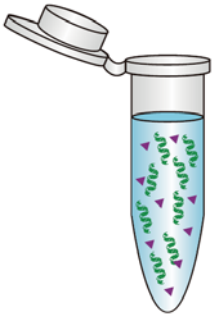
Beside the ready-to-use solutions supplied with this kit, you will need to prepare the following working solution:

Content	Preparation	Storage/ Stability
Lysozyme / DM1 Buffer (White Cap)	Add all 20 µl of Lysozyme into the DM1 Buffer bottle. * Label bottle accordingly after adding Lysozyme.	Store the mixture at +2 to +8°C. Stable for 6 months.
Wash Buffer (Blue Cap)	Add 12 ml absolute ethanol to Wash Buffer. * Label bottle accordingly after adding ethanol.	Store at +15 to +25°C. Stable through expiration date printed on kit label.

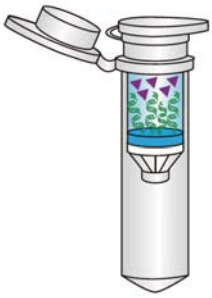
Quick Protocol Diagram



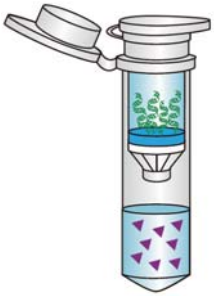
Bacterial pellet suspension



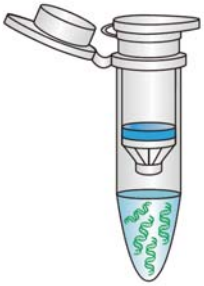
Lysis by Lysozyme and Proteinase K



DNA Binding



Wash



Elution

Genomic DNA Extraction Kit from Gram Positive Bacteria Protocol

1. Pellet the bacterial cells from 1-4 ml of bacterial culture by centrifuge in 8000-10000 g for 1 min. Discard the supernatant.

2. Add 200 µl **DM1 Buffer** (**White Cap**) with Lysozyme to the bacterial pellet. Resuspend the bacterial pellet by vortex for 15 s.

3. Incubate at 37ºC for 30 minutes. During incubation, invert the tube every occasionally.

4. Add 20 µl of Proteinase K then mix by vortex. Incubate at 60ºC for at least 10 minutes. During incubation, invert the tube every 3 minutes.

5. Add 200 µl of **DM2 Buffer** (**Brown Cap**) to the sample and mix by vortex for 10 seconds. Incubate at 70ºC for at least 10 minutes to ensure the sample lysate is clear. During incubation, invert the tube every 3 minutes.

6. Following 70ºC incubation, add 2 µl of RNase A (20 mg/ml) to the clear lysate then shake vigorously. Incubate at room temperature for 5 minutes.

7. Add 200 µl of **absolute ethanol** to the lysate then immediately mix by shaking vigorously for 10 seconds. If precipitate appears, break it up as much as possible with a pipette.

8. Transfer the mixture (including any precipitate) into a **DNA spin column** assembled in a clean collection tube (provided). Centrifuge at 10000 g for 2 min at room temperature.

9. Disconnect the **DNA spin column** from collection tube and discard the flow through solution. Reconnect the **DNA spin column** to the same collection tube.

10. Add 500 µl **Wash Buffer** (**Blue Cap**) to DNA spin column and centrifuge at 10000 g for 1 min. Discard flow through.

11. Centrifuge the **DNA spin column** at 10000 g for 1 min to remove residual ethanol.

12. Place the **DNA spin column** into the clean new microtube. Add 30 µl of pre-warmed **Elution Buffer** (**Black Cap**) or sterile DDW directly onto column membrane and stand for 3 min.

13. Centrifuge at 10000 g for 1 min.

14. Add another 30 µl pre-warmed **Elution Buffer** (**Black Cap**) or sterile DDW directly onto column membrane and stand for 3 min.

15. Centrifuge at 10000 g for 1 min.

16. Check 5-10 µl of extracted DNA by 1% agarose gel electrophoresis.

17. Store DNA at 4˚C or -20˚C.

Related DNA Extraction Products

Product	Package Size	Catalogue Number
Plasmid DNA Isolation Kit	25 preps	DV01025
Plasmid DNA Isolation Kit	50 preps	DV01050
Plasmid DNA Isolation Kit	100 preps	DV01100
DNA Gel Recovery Kit (PCR Purification Kit)	25 prep	DR02025
DNA Gel Recovery Kit (PCR Purification Kit)	50 preps	DR02050
Genomic DNA Extraction Kit from Gram Negative Bacteria	25 prep	DM04025
Genomic DNA Extraction Kit from Gram Negative Bacteria	50 prep	DM04050
Genomic DNA Extraction Kit from Gram Positive Bacteria	25 prep	DM05025
Genomic DNA Extraction Kit from Gram Positive Bacteria	50 prep	DM05050
Genomic Yeast DNA Extraction Kit	25 prep	DY06025
Genomic Yeast DNA Extraction Kit	50 prep	DY06050
Plant Total DNA Extraction Kit	50 prep	DP07050
Plant Total DNA Extraction Kit	100 prep	DP07100
Genomic DNA Extraction Kit from Seed	50 prep	DS08050
Genomic Fungi DNA Extraction Kit	25 prep	DF09025
Genomic Fungi DNA Extraction Kit	50 prep	DF09050
Blood DNA Extraction Kit	25 prep	DB10025
Blood DNA Extraction Kit	50 prep	DB10050
Blood DNA Extraction Kit	100 prep	DB10100
Tissue & Cells DNA Isolation Kit	50 prep	DT11050

www.irgtp.com
Tel: 021 – 88200114
021 – 88200112