



## OmniPrep™ for Gram Positive Bacteria

*For High Quality Genomic DNA Extraction from Gram Positive Bacteria*

### INTRODUCTION

The *OmniPrep™ for Gram Positive Bacteria* kit isolates high quality genomic DNA from Gram-positive bacteria. The kit isolates high purity ( $A_{260}/A_{280}$  ratios of 1.7 to 2) DNA between 100-200kbp and the yield is 25-50µg/ml Gram-positive culture. If used according to the protocols this kit purifies DNA from 100ml Gram-positive bacteria culture.

### ITEM(S) SUPPLIED Cat. # 786-398

Genomic Lysis Buffer	100ml
EDTA (0.5M)	10ml
DNA Stripping Solution	10ml
Precipitation Solution	30ml
<i>LongLife™</i> RNase (5mg/ml; >60U/mg)	0.5ml
<i>LongLife™</i> Lysozyme (1,500U/ul)	5 x 2ml
<i>LongLife™</i> Proteinase K (5mg/ml)	2 x 0.5 ml
TE Buffer	20ml

### STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store the kit components as recommended on the label.

### REAGENTS NOT SUPPLIED WITH THIS KIT

- Chloroform
- Isopropanol and
- 70% Ethanol

### PREPARATION BEFORE USE

*Proteinase K Solution:* To avoid repeated freezing-thaw, dispense the Proteinase K solution into aliquots of 30µl/tube and freeze at -20°C.

*Genomic Lysis Buffer & DNA Stripping Solution:* If a precipitate forms due to cold storage allow to warm to room temperature until precipitate dissolves.

### PROTOCOL FOR GRAM POSITIVE BACTERIA

1. Aliquot 0.5ml Gram-positive bacteria overnight culture into a 1.5ml microfuge tube and centrifuge at 14,000xg for 30 seconds. Discard the supernatant.
2. Add 450µl sterile water and 50µl EDTA to the pellet and gently vortex to resuspend.
3. Add 50µl *LongLife™* Lysozyme, invert to mix and incubate at 37°C for 45 minutes with periodic mixing.
4. Centrifuge for 5 minutes at 14,000xg and pour off the supernatant. Gently vortex the tube to resuspend the pellet in the residual liquid.



5. Add 500µl Genomic Lysis Buffer and mix by inverting the tube several times. Do not vortex.
6. Incubate the sample at 55-60°C for 15 minutes. Do not heat higher than 60°C  
***OPTIONAL:** For maximum DNA recovery, add 1µl Proteinase K solution for every 100µl Lysis Buffer and incubate at 60°C for 1-2 hours. Invert the tube periodically each hour. This step will digest hard to handle tissues and significantly improve the yield.*
7. Allow the sample to cool to room temperature. Add 200µl chloroform and mix by inverting the tube several times. Centrifuge for 10 minutes at 14,000xg and carefully remove the upper phase to a clean microcentrifuge tube.
8. Add 50µl DNA Stripping Solution to the sample and invert several times to mix. Incubate the sample for 5-10 minutes at 60°C.
9. Add 100µl Precipitation Solution and mix by inverting the tube several times. A white precipitate should be produced, if not add 50µl aliquots of Precipitation Solution until a white precipitate forms.
10. Centrifuge the sample at 14,000xg for 5 minutes.
11. Transfer the supernatant to a clean tube and precipitate the genomic DNA with 500µl isopropanol. Invert the tubes 10 times to precipitate the DNA.
12. Centrifuge at 14,000xg for 5 minutes to pellet genomic DNA. Remove the supernatant.
13. Add 700µl 70% ethanol to the tube and invert several times to wash the DNA pellet. Centrifuge for 1 minute at 14,000xg. *In some samples, the pellet may be hard to see at this point and will be loosely attached to the tube.*
14. Decant or pipette off the ethanol wash. Invert the tube on a clean absorbent surface for several minutes to allow any excess ethanol to drain away. Do not let the pellet dry completely or it will be difficult to rehydrate.
15. Add 50 to 100µl TE Buffer to the pellet. Incubate at room temperature for at least 15 minutes to rehydrate. Incubating the tube at 55-60°C will speed up rehydration. Incubate for 5-60minutes.  
***OPTIONAL:** 1µl LongLife™ RNase for every 100µl TE Buffer can be added at this stage.*
16. Store DNA at 4°C, for long-term storage store at -20°C or -80°C.

#### **RELATED PRODUCTS**

1. **MegaLong-DNA (Cat#786-136)**: For isolation of genomic DNA >100kb.
2. **OmniTemplate-DNA (Cat# 786-013)**: A single tube method for genotyping and large-throughput applications.
3. **RapidTemplate-DNA (Ca. #786-015)**: For isolation of genomic DNA from small samples low in DNA.

**NOTE:** For other related products, visit our web site at [www.GBiosciences.com](http://www.GBiosciences.com) or contact us.