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A Geno Technology, Inc. (USA) brand name

XIT™ Genomic DNA from Gram Positive Bacteria

For the Isolation of Genomic DNA from Bacterial Overnight Cultures

(Cat. # 786-339, 786-340)

INTRODUCTION

The XIT™ Genomic DNA from Gram Positive Bacteria kit is designed for the isolation of genomic DNA from Gram positive bacteria cultures. The XIT™ kit uses lytic digestion of cell walls, cell lysis, protein precipitation and finally DNA precipitation to isolate high quality genomic DNA.

XIT™ Genomic DNA from Gram Positive Bacteria kit is for the processing of a maximum of 25 or 250ml of culture. XIT™ Genomic DNA from Gram Positive Bacteria Kit protocol is designed to use 1ml overnight culture, however the protocol can be easily adapted for larger tissue sample sizes. The purified DNA has an A_{260}/A_{280} ratio between 1.7 and 1.9, and has yields ranging between 15-25µg/ml depending on culture density.

ITEM(S) SUPPLIED

Description	Cat # 786-339 For 25ml Culture	Cat # 786-340 For 250ml Culture
XIT™ Cell Suspension Solution	10ml	100ml
LongLife™ Lysozyme	1ml	2 x 1ml
XIT™ Lysis Buffer	10ml	100ml
XIT™ Protein Precipitation Buffer	2.5ml	25ml
TE Buffer	1.5ml	20ml
LongLife™ RNase	0.5ml	0.5ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store the LongLife™ Lysozyme and LongLife™ RNase at -20°C, all other kit components at room temperature. The kit components are stable for 1 year, if stored properly.

ADDITIONAL ITEMS REQUIRED

Isopropanol, 70% ethanol

PREPARATION BEFORE USE

1. Preheat a waterbath or heating block to 37°C.
2. Equilibrate TE Buffer to 50-60°C.

PROTOCOL

1. Remove 1ml of overnight bacterial culture (~0.5-2x10^9 cells) and transfer to a 1.5ml centrifuge tube.
2. Centrifuge tube at 5,000xg for 5 minute to pellet bacteria.
3. Add 400µl XIT™ Cell Suspension Solution to cell pellet and gently pipette up and down until cells are suspended.

4. Add 5µl LongLife™ Lysozyme to the tube and mix by inverting the tube 10-20 times. Incubate at 37°C for 30 minutes. Invert the tube periodically during the incubation.
5. After incubation, centrifuge the tube at 14,000g for 1 minute to pellet the spheroplasts.
6. Add 400µl XIT™ Lysis Buffer and pipette up and down to lysate the bacterial spheroplasts.
   NOTE: *For some species, an incubation at 80°C for 5 minutes may required for improved lysis.*
7. Add 90µl XIT™ Protein Precipitation Buffer to the sample and mix by inverting the tube 10-20 times.
8. Centrifuge at 14,000xg for 5 minutes. Carefully, transfer the supernatant to a new tube.
   NOTE: *The supernatant should be clear. If not, repeat the centrifugation.*
9. Add 400µl isopropanol to the supernatant and mix by gently inverting the sample at least 20-25 times.
10. Centrifuge at 14,000rpm for 5 minutes.
11. Discard the supernatant and use a pipette to carefully remove remaining liquid without disturbing the pellet.
12. Add 200µl 70% ethanol and invert the tube twice to wash the pellet.
13. Centrifuge at 14,000rpm for 5 minutes.
14. Discard the supernatant and drain the tube on a piece of clean absorbent paper. Allow to air dry for 15 minutes.
15. Add 50µl prewarmed TE buffer (or DI H2O) and 1µl LongLife™ RNase to remove the RNA (if required).
16. Rehydrate the genomic DNA by incubating at 55-65°C for one hour, followed by an overnight incubation at room temperature to ensure complete genomic DNA hydration.
17. Store DNA at 4°C, for long term storage store at -20 or -80°C.

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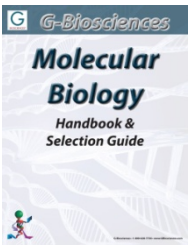

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