G-Biosciences, St Louis, MO. USA ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

XIT™ Genomic DNA from Gram Negative Bacteria

For the isolation of genomic DNA from bacteria

INTRODUCTION

The *XIT*™ Genomic DNA kit is designed for the isolation of genomic DNA from Gram negative bacteria cultures. The *XIT*™ Genomic kit uses the principle of cell lysis, protein precipitation and finally DNA precipitation to isolate high quality genomic DNA.

The *XIT*™ Genomic DNA from Gram Negative Bacteria kit is for the processing of a maximum of 25 or 250ml of culture. *XIT*™ Genomic DNA from Gram Negative 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 1.8-2.0 and has yields ranging between 20-40µg/ml depending on culture density.

ITEM(S) SUPPLIED	Cat # 786-337 *For 25ml Culture*	Cat # 786-338 *For 250ml Culture*
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*™ RNase at -20°C, all other kit components at room temperature. The kit components are stable for 1 year, if stored and used properly.

ITEMS NEEDED BUT NOT SUPPLIED

Isopropanol, 70% ethanol

PREPARATION BEFORE USE

I. Preheat a water-bath or heating block to 80°C.

II. Equilibrate TE Buffer to 50-60°C.

III. Equilibrate *XIT*™ Lysis Buffer to room temperature before use.

IV. Equilibrate *XIT*™ Protein Precipitation Buffer to room temperature before use.

PROTOCOL

1. Remove 1-3ml of overnight culture (~1-4x10^9 cells) and transfer to a 1.5ml centrifuge tube.
2. Centrifuge tube at 5,000g for 2 minutes to pellet Gram-negative bacteria. Discard the supernatant.
3. Add 400µl *XIT*™ Lysis Buffer and pipette up and down several times to lyse the bacteria.
4. Incubate at room temperature for 5-10 minutes.
5. Add 90µl *XIT*™ Protein Precipitation Buffer to the sample and mix by inverting the tube 10-20 times.
6. Centrifuge at 14,000g for 5 minutes. Carefully, transfer the supernatant to a fresh tube.

   NOTE: The supernatant should be clear. If not, repeat the centrifugation.
7. Add 400µl isopropanol to the supernatant and mix by gently inverting the sample at least 20-25 times.

8. Centrifuge at 14,000rpm for 10 minutes.
9. Discard the supernatant and use a pipette to carefully remove remaining liquid without disturbing the pellet.
10. Add 200µl 70% ethanol and invert the tube twice to wash the pellet.
11. Centrifuge at 14,000rpm for 5 minutes.
12. Discard the supernatant and drain the tube on a piece of clean absorbent paper. Allow to air dry for 15 minutes.
13. Add 50µl TE buffer and 1µl *LongLife*™ RNase to remove the RNA (if required).
14. 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.
15. Store DNA at 4°C for short term storage and for long term storage store it at -20 or -80°C as per the requirement.

RELATED PRODUCTS

1. ***EZ-Grind™ (Cat # 786-139):*** *A highly efficient grinding resin that is pre-aliquoted into 1.5ml grinding tubes and is supplied with matching pestles.*
2. ***Pestle & Tubes (Cat. # 786-138P):*** *DNase/RNase free microfuge tubes (1.5ml) and matching pestles for the grinding of small samples and isolation of nuclei.*
3. ***Molecular Grinding Resin™ (Cat # 786-138):*** *For grinding of small samples. High tensile micro particles that do not bind nucleic acids, allowing most samples to be processed by hand using inexpensive micro centrifuge tube pestles or a mortar and pestle.*

NOTE: *For other related products, visit our web site at www.GBiosciences.com or contact us.*

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