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A Geno Technology, Inc. (USA) brand name

XIT™ Genomic DNA from Yeast

For the Isolation of Genomic DNA From Yeast Overnight Cultures

(Cat. # 786-348, 786-349)

INTRODUCTION

The XIT™ Genomic DNA for Yeast kit is designed for the isolation of genomic DNA from yeast cultures. The XIT™ kit uses the principle of lytic digestion of cell walls, cell lysis, protein precipitation and finally DNA precipitation to isolate high quality genomic DNA. XIT™ Genomic DNA from Yeast kit is for the processing of a maximum of 25 or 250ml of culture. XIT™ Genomic DNA from Yeast 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 1-6µg/ml depending on culture density.

ITEM(S) SUPPLIED

Description	Cat # 786-348 For 25ml Culture	Cat # 786-349 For 250ml Culture
XIT™ Cell Suspension Buffer	10ml	100ml
XIT™ Lysis Buffer	10ml	100ml
LongLife™ Zymolyase®	0.5ml	3 x 0.5ml
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™ Zymolyase® and LongLife™ RNase at -20°C, all other kit components at room temperature. The kit components are stable for 1 year, if stored properly.

ITEMS NEEDED BUT NOT SUPPLIED

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 yeast culture (~1-2x10^8 cells) and transfer to a 1.5ml centrifuge tube.
2. Centrifuge tube at 5,000g for 5 minutes to pellet yeast.
3. Resuspend the yeast pellet in 400µl XIT™ Cell Suspension Buffer
4. Add 5µl LongLife™ Zymolyase® to the tube and mix by inverting the tube 10-20 times. Incubate at 37°C for 30 minutes with periodic inversion of the tube.
5. After incubation, centrifuge the tube at 5,000g for 5 minute to pellet the spheroplasts.
6. Add 400µl XIT™ Lysis Buffer and pipette up and down to lyze the yeast spheroplasts.
7. Add 90µl XIT™ Protein Precipitation Buffer to the sample and mix by inverting the tube 10-20 times.
8. Centrifuge at 14,000g for 5 minutes. Carefully, transfer 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 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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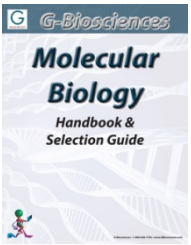

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