XIT™ Genomic DNA from Yeast

For the isolation of genomic DNA from yeast overnight cultures

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	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. Invert the tube periodically during the incubation.
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 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 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.

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.*