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A Geno Technology, Inc. (USA) brand name

XIT™ Genomic DNA from FFPE Tissue

For the isolation of genomic DNA from formalin fixed, paraffin embedded tissue

(Cat. #786-290)

INTRODUCTION

The *XIT*™ Genomic DNA kit is designed for the isolation of genomic DNA from formalin fixed, paraffin embedded tissue. The *XIT*™ kit uses solvent extraction, cell lysis, protein digestion and precipitation and finally DNA precipitation to isolate high quality genomic DNA.

XIT™ Genomic DNA from FFPE Tissue kit is offered for the processing of a maximum of 0.25g of tissue. The purified DNA has a A_{260}/A_{280} ratio between 1.7 and 1.9, and is up to 200kb in size. The yield is 0.5-10µg per mg solid tissue.

ITEM(S) SUPPLIED (Cat. # 786-290)

Description	Size
XIT™ Lysis Buffer	10ml
LongLife™ Proteinase K	0.5ml
XIT™ Protein Precipitation Buffer	2.5ml
Mussel Glycogen Solution	50µl
TE Buffer	1.5ml
LongLife™ RNase	0.5ml

STORAGE CONDITIONS

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

ITEMS NEEDED BUT NOT SUPPLIED

Isopropanol, 70% ethanol, xylene.

PREPARATION BEFORE USE

1. Read appropriate protocol and preheat waterbaths or heating blocks to appropriate temperatures.
2. Equilibrate TE Buffer to 50-60°C.

PROTOCOL FOR FFPE FIXED TISSUE

1. Final chop <10mg formaldehyde fixed paraffin embedded (FFPE) tissue and transfer to a 1.5ml centrifuge tube.
2. Transfer 400µl xylene to the tube and incubate at room temperature with gentle shaking for 5 minutes.
   NOTE: Wear gloves, safety goggles and lab coat when using xylene.
3. Centrifuge the tube at 14,000g for 3 minutes to pellet the tissue. Carefully discard the supernatant.
4. Repeat steps 2 and 3 two more times.
5. Resuspend the tissue in 400µl 90% ethanol and incubate at room temperature with gentle shaking for 5 minutes.
6. Centrifuge the tube at 14,000g for 3 minutes to pellet the tissue. Carefully discard the supernatant.
7. Repeat steps 5 and 6.
8. Transfer 400µl *XIT*™ Lysis Buffer to the tissue. Homogenize the sample until a homogeneous solution is obtained.
   NOTE: *For efficient grinding, we recommend G-Biosciences' EZ-Grind*™ *(Cat. # 786-139), a high efficient grinding resin with matching pestle and tubes.*
9. Add 10µl *LongLife*™ Proteinase K to the tube and mix by inverting the tube 20 times. Incubate at 55°C overnight for maximal yield. Invert the tube periodically during the incubation.
10. If tissue is not completely digested, add a further 10µl *LongLife*™ Proteinase K and incubate at 55°C for 3 hours. Invert the tube periodically during the incubation.
11. Add 90µl *XIT*™ Protein Precipitation Buffer to the sample and mix by inverting the tube 10-20 times.
12. Centrifuge at 14,000g for 5 minutes. Carefully, transfer the supernatant to a fresh tube.
    NOTE: The precipitated protein should form a tight white pellet. If not, incubate the sample on ice for 5 minutes and repeat the centrifugation.
13. Add 400µl isopropanol to the supernatant and mix by gently inverting the sample 30-50 times.
    NOTE: If DNA concentrations is expected to be low (<10µg), add 1µl Mussel Glycogen Solution.
14. Centrifuge at 14,000g for 5 minutes.
15. Discard the supernatant and use a pipette to carefully remove excess liquid.
16. Add 200µl 70% ethanol and invert the tube twice to wash the pellet.
17. Centrifuge at 14,000g for 2 minutes.
18. Discard the supernatant and drain the tube on a piece of clean absorbent paper. Allow to air dry for 15 minutes.
19. Add 50µl prewarmed TE buffer and 1µl *LongLife*™ RNase to remove the RNA (if required).

20. 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.
21. Store DNA at 4°C, for long term storage store at -20 or -80°C

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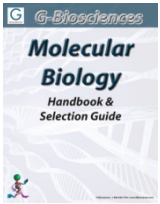

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