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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)



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INTRODUCTION

The XIT^{T} Genomic DNA kit is designed for the isolation of genomic DNA from formalin fixed, paraffin embedded tissue. The XIT^{T} 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.

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

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

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store the *LongLife*^{\mathbb{T}} Proteinase K and *LongLife*^{\mathbb{T}} 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 400μ l 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 400μ l 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.
- 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.
- 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.
- 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.

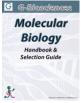
NOTE: The precipitated protein should form a tight white pellet. If not, incubate the sample on ice for 5 minutes and repeat the centrifugation.

- 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 $LongLife^{T}$ 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

RELATED PRODUCTS

Download our Molecular Biology Handbook.



http://info2.gbiosciences.com/complete-molecular-biology-handbook For other related products, visit our website at <u>www.GBiosciences.com</u> or contact us.

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