XIT™ Genomic DNA from Tissue

For the isolation of genomic DNA from fresh or frozen tissue

INTRODUCTION

The *XIT*™ Genomic DNA kit is designed for the isolation of genomic DNA from fresh or frozen mammalian tissue. The *XIT*™ kit uses cell lysis, protein digestion and precipitation and finally DNA precipitation to isolate high quality genomic DNA. The kit is supplied with separate protocols for the following conditions:

1. 1-10mg fresh or frozen tissue
2. 50-100mg of fresh or frozen tissue
3. Fixed Tissue

XIT™ Genomic DNA from Tissue kits are offered for the processing of a maximum of 0.25, 2.5 and 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 # 786-345 *For 250mg tissue*	Cat # 786-346 *For 2.5g tissue*	Cat # 786-347 *For 10g tissue*
XIT™ Lysis Buffer	10ml	100ml	2 x 200ml
LongLife™ Proteinase K	0.5ml	12.5ml	50ml
XIT™ Protein Precipitation Buffer	2.5ml	25ml	100ml
Mussel Glycogen Solution	50µl	1ml	2 x 1ml
TE Buffer	1.5ml	20ml	60ml
LongLife™ RNase	0.5ml	0.5ml	1ml

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

PREPARATION BEFORE USE

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

I. PROTOCOL FOR 1-10mg TISSUE

1. For optimal yield, freeze 1-10mg tissue in liquid nitrogen and quickly grind in liquid nitrogen with a pestle and mortar. Keep the tissue on ice at all times.

   NOTE: *If liquid nitrogen is not available, freeze the tissue and rapidly grind or homogenize on ice in the presence of 200µl XIT*™ *Lysis Buffer. Once thoroughly homogenized add a further 200µl XIT*™ *Lysis Buffer. Proceed to step 3.*

   NOTE: *For efficient grinding, we recommend G-Biosciences' EZ-Grind*™ *(Cat. # 786-139), a high efficient grinding resin with matching pestle and tubes.*

2. Transfer the ground or homogenized tissue to a 1.5ml microfuge tube and add 400µl *XIT*™ Lysis Buffer. If large clumps are visible grind the tissue further in the presence of the lysis buffer.

3. Add 10µl *LongLife*™ Proteinase K to the tube and mix by inverting the tube 10-20 times. Incubate at 55°C for at least 2 hours. The incubation can be incubated overnight for maximal yield. Invert the tube periodically during the incubation.

   NOTE: *Treatment with LongLife™ Proteinase K ensures optimal recovery of genomic DNA, however if time is a factor, the LongLife™ Proteinase K can be omitted and the sample heated at 65°C for 1 hour.*
4. After incubation, incubate the sample on ice for 1 minute to quickly cool. Do not store on ice.
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 2 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.*
7. 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 (<1µg), add 1µl Mussel Glycogen Solution.*
8. Centrifuge at 14,000g for 5 minutes.
9. Discard the supernatant and use a pipette to carefully remove excess liquid.
10. Add 200µl 70% ethanol and invert the tube twice to wash the pellet.
11. Centrifuge at 14,000g for 2 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 prewarmed 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 long term storage store at -20 or -80°C.

II. PROTOCOL FOR 50-100mg TISSUE

1. For optimal yield, freeze 50-100mg tissue in liquid nitrogen and quickly grind in liquid nitrogen with a pestle and mortar. Keep the tissue on ice at all times.

   NOTE: *If liquid nitrogen is not available, freeze the tissue and rapidly grind or homogenize on ice in the presence of 2ml XIT™ Lysis Buffer. Once thoroughly homogenized add a further 2ml XIT™ Lysis Buffer and transfer to a 15ml centrifuge tube. Proceed to step 3.*
2. Transfer the ground or homogenized tissue to a 15ml centrifuge tube and add 4ml *XIT*™ Lysis Buffer. If large clumps are visible grind the tissue further in the presence of the lysis buffer.
3. Add 200µl *LongLife*™ Proteinase K to the tube and mix by inverting the tube 10-20 times. Incubate at 55°C for at least 2 hours. The incubation can be incubated overnight for maximal yield. Invert the tube periodically during the incubation.

   NOTE: Treatment with LongLife™ Proteinase K ensures optimal recovery of genomic DNA, however if time is a factor, the LongLife™ Proteinase K can be omitted and the sample heated at 65°C for 1 hour.
4. After incubation, incubate the sample on ice for 1 minute to quickly cool.
5. Add 900µl *XIT*™ Protein Precipitation Buffer to the sample and mix by inverting the tube 10-20 times.
6. Centrifuge at 2,000-5,000g for 10 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.
7. Add 4ml isopropanol to the supernatant and mix by gently inverting the sample 30-50 times.
8. Centrifuge at 2,000-5,000g for 5 minutes.
9. Discard the supernatant and use a pipette to carefully remove excess liquid.

10. Add 1ml 70% ethanol and invert the tube twice to wash the pellet.
11. Centrifuge at 2,000-5,000g 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 150µl prewarmed TE buffer and 3µl *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 long term storage store at -20 or -80°C.

III. PROTOCOL FOR FIXED TISSUE

1. Transfer 400µl *XIT*™ Lysis Buffer to a clean 1.5ml microfuge tube.
2. Blot excess fixative from tissue and transfer 5-10mg fixed tissue into the *XIT*™ Lysis Buffer. Incubate at 65°C for 15-30 minutes.
3. Homogenize the softened tissue with ~50 strokes of a microfuge tube pestle. We recommend G-Biosciences' Pestles and Tubes (Cat. # 786-138P).
4. 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.
5. If tissue is not completely digested, add a further 10µl *LongLife*™ Proteinase K and incubate at 55°C for 3 hours. Invert Invert the tube periodically during the incubation.
6. After incubation, incubate the sample on ice for 1 minute to quickly cool.
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 2 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.*
9. 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.*
10. Centrifuge at 14,000g for 5 minutes.
11. Discard the supernatant and use a pipette to carefully remove excess liquid.
12. Add 200µl 70% ethanol and invert the tube twice to wash the pellet.
13. Centrifuge at 14,000g for 2 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.*

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