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A Geno Technology, Inc. (USA) brand name

XIT™ Genomic DNA from Cells

For the Isolation of Genomic DNA from Cultured Cells

(Cat. # 786-303, 786-304)

INTRODUCTION

The *XIT*™ Genomic DNA kit is designed for the isolation of genomic DNA from fresh or frozen cultured cells. The *XIT*™ kit uses cell lysis, protein digestion and precipitation and finally DNA precipitation to isolate high quality genomic DNA.

XIT™ Genomic DNA from Cells kits are offered for the processing of a maximum of $5x10^7$ or $5x10^8$ cells. 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 5-10µg per $1-2x10^6$ cells.

ITEM(S) SUPPLIED

Description	**Cat. # 786-303 2.5-5x10^7 cells**	**Cat. # 786-304 2.5-5x10^8 cells**
XIT™ Lysis Buffer	10ml	100ml
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 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.

ADDITIONAL ITEM REQUIRED

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.
3. Completely and quickly thaw frozen cells at 37°C and keep all cells on ice until required.
4. Calculate the number of cells using a hemacytometer or other cell culture.

PROTOCOL FOR 1-2 X 10^6 CELLS IN SUSPENSION

1. Centrifuge an appropriate number (1-2x10^6) of cells at 300g for 5 minutes or 14,000g for 5 seconds in a 1.5ml centrifuge tube.
2. Carefully discard the supernatant by pipetting , leaving ~20µl residual liquid. Vigorously vortex the tube to resuspend the cells in the residual supernatant.
3. Add 400µl *XIT*™ Lysis Buffer to the cells and mix by pipetting or vortexing.
   NOTE: *If clumps are visible, incubate the cells at 37°C with intermittent vortexing until a homogenous solution is seen..*
4. Add 90µl *XIT*™ Protein Precipitation Buffer to the sample and mix by inverting the tube 10-20 times.
5. 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.*
6. Add 400µl isopropanol to the supernatant and mix by gently inverting the sample 30-50 times.
7. Centrifuge at 14,000g for 5 minutes.
8. Discard the supernatant and use a pipette to carefully remove excess liquid.
9. Add 200µl 70% ethanol and invert the tube twice to wash the pellet.
10. Centrifuge at 14,000g for 2 minutes.
11. Discard the supernatant and drain the tube on a piece of clean absorbent paper. Allow to air dry for 15 minutes.
12. Add 50µl pre-warmed TE buffer and 1µl *LongLife*™ RNase to remove the RNA (if required).
13. 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.
14. Store DNA at 4°C, for long term storage store at -20 or -80°C.

PROTOCOL FOR 1-2X10^6 ADHERENT CELLS

1. Remove the cell culture medium and wash cells with PBS. Remove the PBS and add 0.1-0.25% trypsin solution.
2. After the cells have detached, collect the cells in medium and calculate the cell number.
3. Centrifuge an appropriate number (1-2x10^6) of cells at 300g for 5 minutes or 14,000g for 5 seconds in a 1.5ml centrifuge tube.
4. Carefully discard the supernatant by pipetting , leaving ~20µl residual liquid. Vigorously vortex the tube to resuspend the cells in the residual supernatant.
5. Add 400µl *XIT*™ Lysis Buffer to the cells and mix by pipetting or vortexing.
   NOTE: *If clumps are visible, incubate the cells at 37°C with intermittent vortexing until a homogenous solution is seen..*
6. Add 90µl *XIT*™ Protein Precipitation Buffer to the sample and mix by inverting the tube 10-20 times.
7. 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.*
8. Add 400µl isopropanol to the supernatant and mix by gently inverting the sample 30-50 times.
9. Centrifuge at 14,000g for 5 minutes.
10. Discard the supernatant and use a pipette to carefully remove excess liquid.
11. Add 200µl 70% ethanol and invert the tube twice to wash the pellet.
12. Centrifuge at 14,000g for 2 minutes.
13. Discard the supernatant and drain the tube on a piece of clean absorbent paper. Allow to air dry for 15 minutes.
14. Add 50µl pre-warmed TE buffer and 1µl *LongLife*™ RNase to remove the RNA (if required).
15. 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.
16. Store DNA at 4°C, for long term storage store at -20 or -80°C.

PROTOCOL FOR 1-2X10^7 CELLS

1. Follow procedure for cell suspension or adherent cells until the number of cells have been calculated.
2. Centrifuge an appropriate number (1-2x10^7) of cells at 300g for 5 minutes or 14,000g for 5 seconds in a 15ml centrifuge tube.
3. Carefully discard the supernatant by pipetting , leaving ~200µl residual liquid. Vigorously vortex the tube to resuspend the cells in the residual supernatant.
4. Add 4ml *XIT*™ Lysis Buffer to the cells and mix by pipetting or vortexing.
   NOTE: *If clumps are visible, incubate the cells at 37°C with intermittent vortexing until a homogenous solution is seen.*
5. Add 900µl *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 4ml isopropanol to the supernatant and mix by gently inverting the sample 30-50 times.
8. Centrifuge at 14,000g for 5 minutes.
9. Discard the supernatant and use a pipette to carefully remove excess liquid.
10. Add 2ml 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 250µl pre-warmed TE buffer and 5µl *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.

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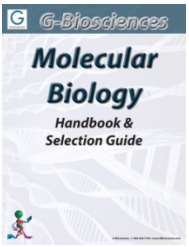

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