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A Geno Technology, Inc. (USA) brand name

XIT™ Genomic DNA Blood

For the Isolation of Genomic DNA from Fresh Blood, Bone Marrow & Buffy Coat

(Cat. # 786-294, 786-295, 786-296)

INTRODUCTION

The XIT™ Genomic DNA Blood kits are designed for the isolation of genomic DNA from whole blood, bone marrow and buffy coat. The XIT™ kit uses the principle of cell lysis, protein precipitation and finally DNA precipitation to isolate high quality genomic DNA.

XIT™ Genomic DNA Blood Kit protocol is designed to use 0.5ml whole blood, 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 10-15µg/ml depending on volume of blood.

ITEM(S) SUPPLIED

Description	**Cat # 786-294** *≤12.5ml blood*	**Cat # 786-295** *≤125ml blood*	**Cat # 786-296** *≤250ml blood*
RBC Lysis Buffer	100ml	2 x 250ml	4 x 250ml
XIT™ Lysis Buffer	10ml	100ml	2 x 100ml
XIT™ Protein Precipitation Buffer	2.5ml	25ml	2 x 25ml
TE Buffer	1.5ml	20ml	2 x 20ml
LongLife™ RNase	0.5ml	0.5ml	2 x 0.5ml

STORAGE CONDITIONS

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

ADDITIONAL ITEMS REQUIRED

- Isopropanol
- 70% ethanol

PREPARATION BEFORE USE

1. Preheat a water-bath or heating block to 55°C and equilibrate TE Buffer to 50-60°C.

PROTOCOL

1. For processing buffy coats, use the volumes required for processing the original blood sample. For example, if the buffy coat preparation was processed from 5ml whole blood then follow the Protocol for 5ml Blood.
2. For bone marrow samples, ensure that the sample is completely homogenous after addition of *XIT*™ Lysis Buffer. If not add additional *XIT*™ Lysis Buffer until an homogenous sample is obtained.

FOR 0.5ml BLOOD

1. Add 0.5ml whole blood to a 1.5ml tube containing 1ml RBC Lysis Buffer. Invert the tube to mix and incubate 2-3 minutes at room temperature.
2. Centrifuge 14,000xg for 30 seconds then remove supernatant carefully without disturbing the pellet.
3. Add 1ml of RBC Lysis Buffer to the pellet and mix.
4. Centrifuge 14,000xg for 30 seconds then remove supernatant. Repeat step 3 and 4 if pellet is not white.
5. Vortex the tube to resuspend the cells in the residual liquid.
6. Add 400µl of *XIT*™ Lysis Buffer to the resuspended cells and vortex vigorously to lyse the cells. Usually no incubation is required; however, if cell clumps are visible after mixing, incubate at 37°C for 5-10 minutes or until the solution is homogenous. ***OPTIONAL****: Add 2µl LongLife*™ *RNase solution to the cell lysate, mix by inverting the tube 10-15 times and incubate at 37°C for 15 minutes.*
7. Add 90µl *XIT*™ Protein Precipitation Buffer to the sample and mix by inverting the tube 10-20 times.
8. Centrifuge at 16,000g 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 DNA 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 TE buffer to dissolve the DNA.
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.

FOR 5ml BLOOD

1. Add 5ml whole blood to a 15ml centrifuge tube containing 5ml RBC Lysis Buffer. Invert the tube to mix and incubate 2-3 minutes at room temperature.
2. Centrifuge 2,000xg for 5 minutes then remove supernatant carefully without disturbing the pellet.
3. Add 10ml of RBC Lysis Buffer to the pellet and mix.
4. Centrifuge 2,000xg for 5 minutes then remove supernatant. Repeat step 3 and 4 if pellet is not white.
5. Vortex the tube to resuspend the cells in the residual liquid.
6. Add 4ml of *XIT*™ Lysis Buffer to the resuspended cells and vortex vigorously to lyse the cells. Usually no incubation is required; however, if cell clumps are visible after mixing, incubate at 37°C for 5-10 minutes or until the solution is homogenous.
   OPTIONAL*: Add 20µl LongLife™ RNase solution to the cell lysate, mix by inverting the tube 10-15 times and incubate at 37°C for 15 minutes.*
7. Add 900µl *XIT*™ Protein Precipitation Buffer to the sample and mix by inverting the tube 10-20 times.
8. Centrifuge at 2,000g for 5 minutes. Carefully, transfer the supernatant to a new tube.
   NOTE*: The supernatant should be clear. If not, repeat the centrifugation.*
9. Add 4ml isopropanol to the supernatant and mix by gently inverting the sample at least 20-25 times.
10. Centrifuge at 2,000g for 5 minutes.
11. Discard the supernatant and use a pipette to carefully remove remaining liquid without disturbing the DNA pellet.
12. Add 2ml 70% ethanol and invert the tube twice to wash the pellet.
13. Centrifuge at 2,000g for 3 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 500µl TE buffer to dissolve the DNA.
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.

APPENDIX 1: PURIFICATION OF DNA FROM AMNIOTIC FLUID

Additional Items Required

- Isopropanol
- 70% ethanol
- Glycogen Solution [20mg/ml]

Protocol

1. Add 1-3ml Amniotic fluid to a 1.5ml centrifuge tube and centrifuge 14,000xg for 5 seconds then remove supernatant carefully without disturbing the pellet.
   NOTE*: If using 3ml, add 1.5ml to the tube, centrifuge and add a second 1.5ml volume.*
2. Remove supernatant leaving 10-20µl residual liquid in the tube.
3. Vortex the tube to resuspend the cells in the residual liquid.
4. Add 400µl of *XIT*™ Lysis Buffer to the resuspended cells and vortex vigorously to lyse the cells. Usually no incubation is required; however, if cell clumps are visible after mixing, incubate at 37°C for 5-10 minutes or until the solution is homogenous.
   OPTIONAL*: Add 2µl LongLife™ RNase solution to the cell lysate, mix by inverting the tube 10-15 times and incubate at 37°C for 15 minutes.*
5. Place the tube on ice for 1 minute to rapidly cool to room temperature.
6. Add 90µl *XIT*™ Protein Precipitation Buffer to the sample and mix by inverting the tube 10-20 times.
7. Centrifuge at 16,000g for 5 minutes. Carefully, transfer the supernatant to a new tube.
   NOTE*: The supernatant should be clear. If not, repeat the centrifugation.*
8. Add 400µl isopropanol and 5µl Glycogen Solution to the supernatant and mix by gently inverting the sample at least 20-25 times.
   NOTE*: The glycogen solution improves DNA yields, if expected yields are <20µg.*
9. Centrifuge at 14,000rpm for 5 minutes.
10. Discard the supernatant and use a pipette to carefully remove remaining liquid without disturbing the DNA pellet.
11. Add 200µl 70% ethanol and invert the tube twice to wash the pellet.
12. Centrifuge at 14,000rpm for 5 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 TE buffer to dissolve the DNA.
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.

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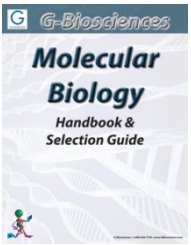

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