

XIT™ Genomic DNA Blood Kit Protocol for Isolation of Genomic DNA (for 5ml blood)

G-Biosciences

Abstract

This is a protocol for the Isolation of Genomic DNA from Fresh Blood, Bone Marrow & Buffy Coat (5ml blood).

If the buffy coat preparation was processed from 5ml whole blood then follow the Protocol for 5ml Blood.

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

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Guidelines

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 A260/A280 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

BUFFY COATS & BONE MARROW SAMPLES

- 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.
- 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.

Before start

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

Materials

XIT™ Genomic DNA from Blood [786-294](#) by [G-Biosciences](#)

Protocol

Step 1.

Add 5ml whole blood to a 15ml centrifuge tube containing 5ml RBC Lysis Buffer.

Step 2.

Invert the tube to mix and incubate 2-3 minutes at room temperature.

 DURATION

00:02:00

Step 3.

Centrifuge 2,000xg for 5 minutes then remove supernatant carefully without disturbing the pellet.

 DURATION

00:05:00

Step 4.

Add 10ml of RBC Lysis Buffer to the pellet and mix.

Step 5.

Centrifuge 2,000xg for 5 minutes then remove supernatant. Repeat steps 4 and 5 if pellet is not white.

 DURATION

00:05:00

Step 6.

Vortex the tube to resuspend the cells in the residual liquid.

Step 7.

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.

 DURATION

00:05:00

 NOTES

Colin Heath 17 Jun 2016

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.

Step 8.

Add 900µl XIT™ Protein Precipitation Buffer to the sample and mix by inverting the tube 10-20 times.

Step 9.

Centrifuge at 2,000g for 5 minutes. Carefully, transfer the supernatant to a new tube.

 DURATION

00:05:00

 NOTES

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NOTE: The supernatant should be clear. If not, repeat the centrifugation.

Step 10.

Add 4ml isopropanol to the supernatant and mix by gently inverting the sample at least 20-25 times.

Step 11.

Centrifuge at 2,000g for 5 minutes.

 DURATION

00:05:00

Step 12.

Discard the supernatant and use a pipette to carefully remove remaining liquid without disturbing the DNA pellet.

Step 13.

Add 2ml 70% ethanol and invert the tube twice to wash the pellet.

Step 14.

Centrifuge at 2,000g for 3 minutes.

 DURATION

00:03:00

Step 15.

Discard the supernatant and drain the tube on a piece of clean absorbent paper. Allow to air dry for 15 minutes.

 DURATION

00:15:00

Step 16.

Add 500µl TE buffer to dissolve the DNA.

Step 17.

Rehydrate the genomic DNA by incubating at 55-65°C for one hour.

 DURATION

01:00:00

Step 18.

Incubate overnight at room temperature to ensure complete genomic DNA hydration.

 DURATION

16:00:00

Step 19.

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