XIT™ Genomic DNA Blood Kit Protocol for Purificaton of DNA from Amniotic Fluid

G-Biosciences

Abstract

This is a protocol for the Isolation of Genomic DNA : PURIFICATION OF DNA FROM AMNIOTIC FLUID

(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

• Glycogen Solution [20mg/ml]

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 1-3ml Amniotic fluid to a 1.5ml centrifuge tube.

Step 2.

Centrifuge 14,000xg for 5 seconds then remove supernatant carefully without disturbing the pellet.

DURATION
00:00:05

NOTES
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NOTE: If using 3ml, add 1.5ml to the tube, centrifuge and add a second 1.5ml volume.

Step 3.

Remove supernatant leaving 10-20µl residual liquid in the tube.

Step 4.

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

Step 5.

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.

DURATION
00:05:00

NOTES
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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.

Step 6.

Place the tube on ice for 1 minute to rapidly cool to room temperature.

DURATION
00:01:00

Step 7.

Add 90µl XIT™ Protein Precipitation Buffer to the sample and mix by inverting the tube 10-20 times.

Step 8.

Centrifuge at 16,000g 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 9.

Add 400µl isopropanol and 5µl Glycogen Solution to the supernatant and mix by gently inverting the sample at least 20-25 times.

NOTES
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NOTE: The glycogen solution improves DNA yields, if expected yields are <20µg.

Step 10.

Centrifuge at 14,000rpm for 5 minutes.

DURATION
00:05:00
Step 11.

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

Step 12.

Add 200µl 70% ethanol and invert the tube twice to wash the pellet.

Step 13.

Centrifuge at 14,000rpm for 5 minutes.

DURATION
00:05:00
Step 14.

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 15.

Add 50µl TE buffer to dissolve the DNA.

Step 16.

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

DURATION
01:00:00

Step 17.

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

DURATION
16:00:00

Step 18.

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