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

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Abstract

This is a protocol for the Isolation of Genomic DNA : PURIFICATION OF DNA FROM AMNIOTIC FLUID
(Cat. # **786-294, 786-295, 786-296**)

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 × 250ml	4 × 250ml
	XIT™ Lysis Buffer	10ml	100ml	2 × 100ml
	XIT™ Protein Precipitation Buffer	2.5ml	25ml	2 × 25ml
	TE Buffer	1.5ml	20ml	2 × 20ml
	LongLife™ RNase	0.5ml	0.5ml	2 × 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.

Materials

MATERIALS

XIT™ Genomic DNA from Blood **G-Biosciences Catalog #**786-294

Troubleshooting

Before start

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

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

2 Centrifuge 14,000xg for 5 seconds then remove supernatant carefully without disturbing the pellet.
00:00:05

Note

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

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

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

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.
00:05:00

Note

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.

6 Place the tube on ice for 1 minute to rapidly cool to room temperature.
00:01:00

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.
00:05:00

Note

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

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

Note

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

10 Centrifuge at 14,000rpm for 5 minutes.

00:05:00

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.

00:05:00

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

00:15:00

15 Add 50µl TE buffer to dissolve the DNA.

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

01:00:00

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

16:00:00

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