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A Geno Technology, Inc. (USA) brand name

OmniPrep™ for Blood

For High Quality Genomic DNA Extraction From Whole Blood, Buffy Coats, Bone Marrow & Packed Cells

(Cat. # 786-396)

INTRODUCTION

The OmniPrep™ for Blood kit isolates high quality genomic DNA from blood samples, including whole blood, buffy coats, packed cells and bone marrow. OmniPrep™ for Blood isolates high purity (A260/A280 ratios of 1.7 to 2) DNA between 100-200kbp and the yield is between 5-30µg/ml, dependent on starting material and quantity.

ITEM(S) SUPPLIED (Cat. # 786-396)

Description	Size
Nuclei Isolation Buffer	250ml
Genomic Lysis Buffer	100ml
DNA Stripping Solution	10ml
Precipitation Solution	30ml
Mussel Glycogen (10mg/ml)	1ml
TE Buffer	20ml
Longlife™ RNase (5mg/ml; >60U/mg)	0.5ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store the kit components as recommended on the label.

REAGENTS NOT SUPPLIED WITH THIS KIT

- Isopropanol
- 70% Ethanol
- Chloroform

PREPARATION BEFORE USE

Genomic Lysis Buffer & DNA Stripping Solution: If a precipitate forms due to cold storage allow to warm to room temperature until precipitate dissolves.

PROTOCOL FOR <500µL BLOOD

1. To <500µl whole blood, buffy coat, bone marrow or packed cells in a 2ml microfuge tube, add 0.6ml Nuclei Isolation Buffer. Invert to mix and incubate at room temperature for 1 minute, invert at least twice during incubation.
2. Centrifuge at ~14,000g for 30 seconds to pellet the whole blood cells and nuclei. Remove the supernatant containing lysed red blood cells, retaining the pellet.
3. Vortex to resuspend the pellet and add 0.6ml Nuclei Isolation Buffer. Invert tube to mix and then incubate for 10 minutes at room temperature, inverting the tube every 1-2 minutes.
4. Centrifuge at ~14,000g for 30 seconds to pellet the nuclei. Remove the supernatant and retain the white nuclei pellet with 10-20µl supernatant.
5. Vortex to resuspend the pellet for improved nuclear lysis and add 500µl Genomic Lysis Buffer. Mix by pipetting or vortexing at high speed for 10 seconds.
6. For complete lysis, incubate the samples at 55-60°C for 10-15 minutes. Do not heat higher than 60°C
7. Allow the sample to cool to room temperature. Add 200µl chloroform and mix by inverting the tube several times. Centrifuge for 10 minutes at 14,000xg and carefully remove the upper phase to a clean microcentrifuge tube.
8. Add 50µl DNA Stripping Solution to the sample and invert several times to mix. Incubate the sample for 5-10 minutes at 60°C.
9. Add 100µl Precipitation Solution and mix by inverting the tube several times. A white precipitate should be produced, if not add 50µl aliquots of Precipitation Solution until a white precipitate forms.
10. Centrifuge the sample at 14,000xg for 5 minutes. A tight reddish brown pellet should form.
11. Transfer the supernatant to a clean tube and precipitate the genomic DNA with 700µl isopropanol. Invert the tubes 10 times to precipitate the DNA.
    OPTIONAL: *For increased DNA recovery, add 2µl Mussel Glycogen as a DNA carrier.*
12. Centrifuge at 14,000xg for 5 minutes to pellet genomic DNA. Remove the supernatant.
13. Add 700µl 70% ethanol to the tube and invert several times to wash the DNA pellet. Centrifuge for 1 minute at 14,000xg. In some samples, the pellet may be hard to see at this point and will be loosely attached to the tube.
14. Decant or pipette off the ethanol wash. Invert the tube on a clean absorbent surface for several minutes to allow any excess ethanol to drain away. Do not let the pellet dry completely or it will be difficult to rehydrate.
15. Add 50 to 100µl TE Buffer to the pellet. Incubate at room temperature for at least 15 minutes to rehydrate. Incubating the tube at 55-60°C will speed up rehydration. Incubate for 5-60minutes.
    OPTIONAL: *1µl LongLife™ RNase for every 100µl TE Buffer can be added at this stage.*
16. Store DNA at 4°C, for long-term storage store at -20°C or -80°C.

PROTOCOL FOR >600µL BLOOD

1. For <5ml whole blood, buffy coat, bone marrow or packed cells use a 15ml conical tube; for 5-12ml whole blood, buffy coat, bone marrow or packed cells use a 50ml conical tube. For volumes >12ml, divide sample to a maximum of 6ml per 50ml tube. (See table below for quick reference guide.)
2. Add 2.5ml Nuclei Isolation Buffer for every 1ml sample. Invert to mix and incubate at room temperature for 5 minutes, invert at least twice during incubation.
3. Centrifuge at ~2,000*g* for 5 minutes to pellet the nuclei. Remove the supernatant and retain the white nuclei pellet with 100-300µl supernatant.
4. Vortex to resuspend the pellet for improved nuclear lysis and add 1ml Genomic Lysis Buffer for every 1ml starting material. Mix by pipetting or vortexing at high speed for 10 seconds.
5. For complete lysis, incubate the samples at 55-60°C for 10-15 minutes. Do not heat higher than 60°C.
6. Allow the sample to cool to room temperature. Add 400µl chloroform and mix by inverting the tube several times. Centrifuge for 10 minutes at 14,000xg and carefully remove the upper phase to a clean microcentrifuge tube.
7. Add 100µl DNA Stripping Solution for every 1ml starting material and invert several times to mix. Incubate the sample for 5-10 minutes at 60°C.
8. Add 200µl Precipitation Solution for every 1ml starting material and mix by inverting the tube several times. A white precipitate should be produced, if not add 100µl aliquots of Precipitation Solution until a white precipitate forms.
9. Centrifuge the sample at 2,000xg for 5 minutes. A tight reddish brown pellet should form.
   ***NOTE**: If tight pellet does not form, then vortex and incubate on ice for 5 minutes before centrifuging.*
10. Transfer the supernatant to a clean same size (15 or 50ml) tube and precipitate the genomic DNA with 1ml isopropanol for every 1ml starting material. Invert the tubes 10 times to precipitate the DNA.
    ***OPTIONAL:** For increased DNA recovery, add 2µl Mussel Glycogen per ml of starting material as a DNA carrier.*
11. Centrifuge at 2,000xg for 5 minutes to pellet genomic DNA. Remove the supernatant.
12. Add 1ml 70% ethanol/ 1ml starting material to the tube and invert several times to wash the DNA pellet. Centrifuge for 1 minute at 2,000xg. In some samples, the pellet may be hard to see at this point and will be loosely attached to the tube.
13. Carefully decant or pipette off the ethanol wash. Invert the tube on a clean absorbent surface for several minutes to allow any excess ethanol to drain away. Do not let the pellet dry completely or it will be difficult to rehydrate.
14. Add 100µl TE Buffer for every 1ml starting material to the pellet. Incubate at room temperature for at least 15 minutes to rehydrate. Incubating the tube at 55-60°C will speed up rehydration. Incubate for 5-60minutes.

OPTIONAL: *1µl LongLife™ RNase for every 100µl TE Buffer can be added at this stage.*

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

Sample Size	**1ml**	**2ml**	**3ml**	**5ml**	**7ml**	**10ml**	**12ml**
Tube Size	15ml	15ml	15ml	50ml	50ml	50ml	50ml
Nuclei Isolation Buffer	2.5ml	5ml	7.5ml	2.5ml	17.5ml	25ml	30ml
Lysis Buffer	1ml	2ml	3ml	5ml	7ml	10ml	12ml
Chloroform	0.4ml	0.8ml	.2ml	2ml	2.8ml	4ml	4.8ml
DNA Stripping Solution	0.1ml	.2ml	0.3ml	0.5ml	0.7ml	1ml	1.2ml
Precipitation Solution	0.2ml	0.4ml	0.6ml	1ml	1.4ml	2ml	2.4ml
Isopropanol	1ml	2ml	3ml	5ml	7ml	10ml	12ml
70% Ethanol	1ml	2ml	3ml	5ml	7ml	10ml	12ml
TE Buffer	0.1ml	0.2ml	0.3ml	0.5ml	0.7ml	1ml	1.2ml

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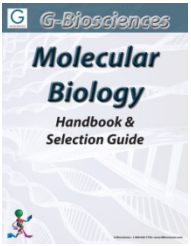

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