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OmniPrep™ For High Quality Genomic DNA Extraction From Blood

In 1 collection

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Abstract

The OmniPrep™ kit isolates high quality genomic DNA from many different species and tissue types including animal, plant, bacteria, yeast, fungi, whole blood, and cells in culture. DNA can be isolated from samples high in polysaccharides or other contaminants that are difficult to remove from the DNA preparations.

This protocol is for use with **blood samples (excluding whole blood)**. Please refer to the **appropriate protocol** depending on your application.

Guidelines

INTRODUCTION

The OmniPrep™ kit isolates high quality genomic DNA from many different species and tissue types including animal, plant, bacteria, yeast, fungi, whole blood, and cells in culture. DNA can be isolated from samples high in polysaccharides or other contaminants that are difficult to remove from the DNA preparations.

Several unique features separate the OmniPrep™ kit from other methods:
• A unique and proprietary formulation of detergents and salts. DNA can be extracted and purified from almost any tissue without the use of toxic agents such as phenol.
• A quick protocol, which isolates extremely clean genomic DNA. On an average, the DNA is 100kb in size and generally hydrates in minutes.
• Provides an option to modify protocol for difficult to handle samples.
• Suitable for 200 Preps (10mg/prep). The kit is adaptable for larger tissue volumes.

ITEM(S) SUPPLIED

	Description	Cat. # 786-136	Cat. # 786-136S
	Genomic Lysis Buffer	100ml	2 × 2ml
	Nuclei Isolation Buffer	2 × 30ml	-
	DNA Stripping Solution	10ml	0.5ml
	Precipitation Solution	30ml	2ml
	Mussel Glycogen (10mg/ml)	1ml	25µl
	TE Buffer	20ml	0.5ml
	Longlife™ RNase (5mg/ml; >60U/mg)	0.5ml	50µl
	Longlife™ Proteinase K (5mg/ml)	2 × 0.5ml	50µl

NOTE: Cat. # 786-136S is a trial/sample size and does not contain enough reagents for all the protocols. For regular size kit, order Cat. # 786-136.

STORAGE CONDITIONS

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

ADDITIONAL REAGENTS REQUIRED

• For all samples: Isopropanol, 70% Ethanol, and Chloroform
• For Gram positive Bacteria: Lysozyme, 0.5M EDTA. Longlife™ Lysozyme, (Cat# 786-037) available.
• For Yeast: Zymolyase® , β-mercaptoethanol, phosphate buffered saline (PBS). Longlife™ Zymolyase® (Cat# 786-036) available.

PREPARATION BEFORE USE

Proteinase K Solution: To avoid repeated freezing-thaw, dispense the Proteinase K solution into aliquots of 30µl/tube and freeze at -20°C.

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

PROTOCOLS

The protocols contained in this manual are listed below and are based on the Extraction from Solid Tissue protocol. Thoroughly review the appropriate protocols before commencing isolation of genomic DNA.

TROUBLESHOOTING

• POOR DNA RECOVERY:

o Increase volumes of Genomic Lysis Buffer, DNA Stripping Solution and Precipitation Solution proportionally.
o Introduce optimal Proteinase K step after step 4.
o Improve grinding technique.
• For efficient grinding of small samples we offer Molecular Grinding Resin™ (Cat. # 786-138), high tensile micro-particles that do not bind nucleic acids and are recommended for grinding and isolation of genomic DNA.
• DNA/RNA free matching pestles and microfuge tubes (1.5ml) for grinding small samples are also available (Cat. # 786-138P).

• FOR >100kb GENOMIC DNA

o Recommend using MegaLong™ DNA isolation kit (Cat. # 786-146, 786-147). Isolates nuclei under mild condition, which are then transferred to Tube-ODIALYZER™ , a new device for DNA isolation. Nuclei are digested with protease followed by dialysis to remove protein and other contaminants. For more information call our Technical Department.

Typical Yield: 5-30µg/ml blood.

LARGER BLOOD VOLUMES

For genomic DNA isolation from larger blood volumes refer to the table below:

	Sample Size	**1ml**	**2ml**	**3ml**	**5ml**	**7ml**	**10ml**	**12ml**
	Tube Size	15ml	15ml	15ml	50ml	50ml	50ml	50ml
	Nuclei Isolation Buffer	3ml	6ml	9ml	15ml	21ml	30ml	36ml

	Lysis Buffer	1ml	2ml	3ml	5ml	7ml	10ml	12ml
	Chloroform	0.4ml	0.8ml	1.2ml	2ml	2.8ml	4ml	4.8ml
	DNA Stripping Solution	0.1ml	0.2ml	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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Materials

MATERIALS

OmniPrep™ **G-Biosciences Catalog #**786-136

Before start

Proteinase K Solution: To avoid repeated freezing-thaw, dispense the Proteinase K solution into aliquots of 30µl/tube and freeze at -20°C.

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

1 To <500µl whole blood, buffy coat, bone marrow or packed cells in a 2ml microfuge tube, add 0.75ml Nuclei Isolation Buffer.

Note

Please see the **Guidelines** for blood samples with volumes above **0.5 ml**.

2 Invert to mix and incubate at room temperature for 1 minute, invert at least twice during incubation.
00:01:00

3 Centrifuge at ~14,000g for 30 seconds to pellet the whole blood cells and nuclei.
00:00:30

4 Remove the supernatant containing lysed red blood cells, retaining the pellet.

5 Vortex to resuspend the pellet and add 0.75ml Nuclei Isolation Buffer.

6 Invert tube to mix and then incubate for 10 minutes at room temperature, inverting the tube every 1-2 minutes.
00:10:00

7 Centrifuge at ~14,000g for 30 seconds to pellet the nuclei.
00:00:30

8 Remove the supernatant and retain the white nuclei pellet with 10-20µl supernatant.

9 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.
00:00:10

10 Incubate the sample at 55-60°C for 15 minutes. Do not heat higher than 60°C.
00:15:00

11 **OPTIONAL**: For maximum DNA recovery, add 1µl Proteinase K solution for every 100µl Lysis Buffer and incubate at 60°C for 1-2 hours. Invert the tube periodically each hour. This step will digest hard to handle tissues and significantly improve the yield.

00:01:00

12 Allow the sample to cool to room temperature.

13 Add 200µl chloroform and mix by inverting the tube several times.

14 Centrifuge for 10 minutes at 14,000xg and carefully remove the upper phase to a clean microcentrifuge tube.
00:10:00

15 Add 50µl DNA Stripping Solution to the sample and invert several times to mix.

16 Incubate the sample for 5-10 minutes at 60°C.
00:05:00

17 Add 100µl Precipitation Solution and mix by inverting the tube several times.

Note

A white precipitate should be produced, if not add 50µl aliquots of Precipitation Solution until a white precipitate forms.

18 Centrifuge the sample at 14,000xg for 5 minutes.
00:05:00

19 Transfer the supernatant to a clean tube and precipitate the genomic DNA with 500µl isopropanol.

20 Invert the tubes 10 times to precipitate the DNA.

21 **OPTIONAL:** For increased DNA recovery, add 2µl Mussel Glycogen as a DNA carrier.

22 Centrifuge at 14,000xg for 5 minutes to pellet genomic DNA.
00:05:00

23 Remove the supernatant.

24 Add 700µl 70% ethanol to the tube and invert several times to wash the DNA pellet.

25 Centrifuge for 1 minute at 14,000xg.
00:01:00

Note

In some samples, the pellet may be hard to see at this point and will be loosely attached to the tube.

26 Decant or pipette off the ethanol wash.

27 Invert the tube on a clean absorbent surface for several minutes to allow any excess ethanol to drain away.

Note

Do not let the pellet dry completely or it will be difficult to rehydrate.

28 Add 50µl TE Buffer to the pellet.

29 Incubate at room temperature for at least 15 minutes to rehydrate.
00:15:00

Note

Incubating the tube at 55-60°C will speed up rehydration. Incubate for 5-60 minutes.

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

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

Note

Typical Yield: 5-30µg/ml blood.