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A Geno Technology, Inc. (USA) brand name

OmniPrep™

For High Quality Genomic DNA Extraction

(Cat. # 786-136, 786-136S)

CONTENTS

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 x 2ml
Nuclei Isolation Buffer	2 x 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 x 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.

1. SOLID TISSUE (FRESH OR FROZEN)

1. For optimal yield, rapidly dissect tissue and proceed with DNA extraction immediately, keeping samples on ice or freeze in liquid nitrogen and store at -70°C until required.
2. On ice, add 1-10mg ground frozen tissue or fresh tissue to a microcentrifuge tube containing 250µl Genomic Lysis Buffer. Homogenize the sample with a microfuge pestle until a homogenous suspension is acquired.
3. Add an additional 250µl Genomic Lysis Buffer.
4. Incubate the sample at 55-60°C for 15 minutes. Do not heat higher than 60°C
   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.*
5. 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.
6. Add 50µl DNA Stripping Solution to the sample and invert several times to mix. Incubate the sample for 5-10 minutes at 60°C.
7. 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.
8. Centrifuge the sample at 14,000xg for 5 minutes.
9. Transfer the supernatant to a clean tube and precipitate the genomic DNA with 500µl isopropanol. Invert the tubes 10 times to precipitate the DNA.
   OPTIONAL: *For increased DNA recovery, add 2µl Mussel Glycogen as a DNA carrier.*
10. Centrifuge at 14,000xg for 5 minutes to pellet genomic DNA. Remove the supernatant.
11. 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.*

12. 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.
13. Add 50µl 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.*
14. Store DNA at 4°C, for long term storage store at -20°C or -80°C.

2. LARGE TISSUE SAMPLES

For 10-50mg tissue perform DNA isolation in a 15ml centrifuge tube, for 50-300mg use a 50ml conical tube. For >300mg tissue, divide sample to a maximum of 300mg per 50ml tube. For other samples, use the same proportions as listed in the table below. Use the same protocol with the following proportions of reagents:

Reagent	Volume per mg tissue	Protocol Step
Genomic Lysis Buffer	25µl	2
Genomic Lysis Buffer	25µl	3
Proteinase K	0.5µl	4
Chloroform	20µl	5
DNA Stripping Solution	5µl	6
Precipitation Solution	10µl	7
Isopropanol	50µl	9
70% Ethanol	50µl	11
TE Buffer	5µl	13

3. CULTURED CELLS

1. Transfer 1-5x10^6 cells, suspension or trypsinized cells into a 1.5ml microfuge tub and add 500µl Genomic Lysis Buffer. Alternatively, lyse cells directly on the culture plate by adding 500µl Genomic Lysis Buffer to 5x10^6 cells. Gently pipette up and down several times to release nuclei from the cells. Transfer lysate to a clean tube.
2. Continue at step 4 of "Extraction from solid tissue" protocol.

Typical yield: *0.2-1.5µg DNA/10^6 Cells*

4. PARAFFIN EMBEDDED TISSUE

1. Finely chop 0.5-2mg paraffin-embedded tissue and place in 1.5ml microfuge tube with 100µl xylene or safe xylene substitute and incubate at room temperature for 5 minutes with constant mixing.
2. Centrifuge at 14,000xg for 2 minutes and discard xylene or xylene substitute. Repeat steps 1-2 twice to achieve a total of three washes.
3. Add 100µl 100% ethanol and incubate for 5 minutes at room temperature with constant mixing.
4. Centrifuge at 14,000xg for 2 minutes and discard ethanol. Repeat steps 3-4 once to achieve a total of two washes.
5. Add 100µl Genomic Lysis Buffer and homogenize the sample with a microfuge pestle until a homogenous suspension is acquired, approximately 30-60 strokes.
6. Incubate the sample at 55-60°C for 15-60 minutes. Do not heat higher than 60°C ***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.*
7. Continue at step 5 of "Extraction from solid tissue" protocol using quantities for 2mg tissue.

Typical Yield: *0.25-1µg DNA /2mg tissue*

5. ETHANOL OR FORMALIN FIXED TISSUE

1. Blot excess fixative from tissue with clean absorbent paper.
2. Add 5-10mg ground frozen tissue or fresh tissue to a microcentrifuge tube containing 500µl Genomic Lysis Buffer and incubate for 15 minutes at 55-65°C to soften tissue.
3. Homogenize the sample with a microfuge pestle until a homogenous suspension is acquired, approximately 30-60 strokes.
4. Incubate the sample at 55-60°C for 15-60 minutes. Do not heat higher than 60°C ***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.*
5. Continue at step 5 of "Extraction from solid tissue" protocol.

Typical Yield: *0.5-10µg DNA/10mg tissue*

6. NUCLEATED BLOOD CELLS FROM BIRD, FISH & FROG

1. Add 10µl nucleated blood to a 1.5ml microfuge tube containing 500µl Lysis Buffer. ***NOTE:*** For larger volume of nucleated blood, use a 15ml tube and add 1ml Lysis Buffer for each 10µl blood sample. Adjust other reagents accordingly, 1ul nucleated blood is equivalent to 1mg tissue in the table on page2.
2. Continue at step 4 of "Extraction from solid tissue" protocol.

Typical Yield: *2-3µg DNA /10^6 Cells*

7. GRAM NEGATIVE BACTERIA

1. Add 0.5 ml of an overnight culture to a 1.5ml microfuge tube.
2. Centrifuge at 16,000xg for 2-3 minutes to pellet the cells. Remove and discard the supernatant. Vortex the tube to re-suspend the cells in residual supernatant.
3. Add 700µl Lysis Buffer and mix by inverting a few times.
4. Continue at step 4 of "Extraction from solid tissue" protocol.

Typical Yield: *30-80µg DNA/1ml Gram negative bacteria.*

8. GRAM POSITIVE BACTERIA

1. Aliquot 0.5ml Gram positive bacteria overnight culture into a 1.5ml microfuge tube and centrifuge at 14,000xg for 30 seconds. Discard the supernatant.
2. Add 450µl sterile water and 50µl EDTA to the pellet and gently vortex to resuspend.
3. Add 50µl *Longlife*™ Lysozyme, invert to mix and incubate at 37°C for 45 minutes with periodic mixing.
4. Centrifuge for 5 minutes at 14,000xg and pour off the supernatant. Gently vortex the tube to resuspend the pellet in the residual liquid.
5. Add 500µl Genomic Lysis Buffer and mix by inverting the tube several times. Do not vortex.
6. Continue at step 4 of "Extraction from solid tissue" protocol.

Typical Yield: *25-50µg/ml Gram positive culture.*

9. PLANT TISSUE (FRESH OR FROZEN)

1. Most plant tissues are best prepared by freezing in liquid nitrogen. Grinding samples in liquid nitrogen to a fine powder and quickly add to an appropriate volume of Lysis Buffer.
2. Add 50-100mg finely ground dried tissue, frozen tissue or fresh leave tissue to a microcentrifuge tube containing 500µl Genomic Lysis Buffer.
3. If ground, vortex for 5 seconds; if unground, homogenize the sample with a microfuge pestle until a homogenous suspension is acquired, approximately 30-60 strokes.
4. Incubate the sample at 65°C for 60 minutes with periodic inversions.
5. Continue at step 5 of "Extraction from solid tissue" protocol.

Typical Yield: 0.5-3µg/mg plant tissue.

10. MOUSE TAIL TISSUE

1. Add 0.5-1cm, approximately 50-100mg, mouse tail in to a 1.5ml microcentrifuge tube with 500µl Genomic Lysis Buffer.
2. Add 10µl Proteinase K solution and incubate at 60°C for 3-4 hours to overnight. Invert the tube periodically if possible.
3. Continue at step 5 of "Extraction from solid tissue" protocol.

Typical Yield: 70-80µg/cm tail.

11. YEAST

1. Aliquot 1.5ml yeast overnight culture into a 1.5ml microfuge tube and centrifuge at 14,000xg for 30 seconds. Discard the supernatant.
2. Add 150µl PBS, 5µl *LongLife™ Zymolyase®* and 1µl β-mercaptoethanol to the pellet and gently vortex to resuspend.
3. Incubate at 37°C for 30 minutes with periodic mixing.
4. Centrifuge for 5 minutes at 14,000xg and pour off the supernatant. Gently vortex the tube to resuspend the pellet in the residual liquid.
5. Add 500µl Genomic Lysis Buffer and mix by inverting the tube several times. Do not vortex.
6. Continue at step 4 of "Extraction from solid tissue" protocol.

Typical Yield: *3-6µg/ml yeast culture.*

12. FUNGAL TISSUE

1. Collect fungal tissue from liquid culture and wash 2-3 times in sterile water.
2. Fungal mycelia are best prepared by grinding samples using Molecular Grinding Resin™ in Genomic Lysis Buffer. For fungal teliospores, grinding samples in liquid nitrogen to a fine powder and quickly add to an appropriate volume of Genomic Lysis Buffer is recommended.
3. Add 10-20mg fungal mycelia to a microcentrifuge tube containing 500µl Genomic Lysis Buffer. Add 30µl Molecular Grinding Resin™ using a wide bore pipette tips and grind with a microcentrifuge pestle. For teliospoes, add ground powder to 500µl Genomic Lysis Buffer and vortex to wet sample.
4. 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.
5. Continue at step 5 of "Extraction from solid tissue" protocol.

Typical Yield: *0.2-1µg/5mg fungal sample.*

13. BLOOD (<0.5ML)

1. To <500µl whole blood, buffy coat, bone marrow or packed cells in a 2ml microfuge tube, add 0.75ml Nuclei Isolation Buffer. Invert to mix and incubate at room temperature for 1 minute, invert at least twice during incubation.
2. Centrifuge at ~14,000*g* 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 0.75ml 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 ~14,000*g* 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. Continue at step 4 of "Extraction from solid tissue" protocol.

Typical Yield: *5-30µg/ml blood.*

14. 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

15. BLOOD STAINED & BODY FLUID STAINED MATERIAL

1. To 10-30mm^2 section of stained material in a 2ml microfuge tube, add 600µl Lysis Buffer and 10µl Proteinase K. Invert to mix.
2. Incubate the sample at 65°C for 4 hours with periodic inversions.
3. Allow to cool to room temperature, remove the stained material and remove excess buffer from the material with a pipette and return to the tube.
4. Continue at step 5 of "Extraction from solid tissue" protocol.

16. BODY FLUIDS

This includes CSF, plasma, saliva, serum, sputum, synovial fluid, urine and whole blood

1. Add 50µl body fluid to a 1.5ml microfuge tube.
   NOTE: For body fluids with a low cell number, concentrate the cells by centrifuging 5-40ml sample at 2,000xg for 10 minutes.
2. For samples with a normal protein concentration, add 250µl Genomic Lysis Buffer and mix by pipetting up and down. For samples with a high protein concentration, add 550µl Genomic Lysis Buffer and mix by pipetting up and down .
3. Continue at step 4 of main protocol.

Typical Yield: *0.2-5µg DNA/100µl body fluid.*

TROUBLESHOOTING

- **POOR DNA RECOVERY:**
  - Increase volumes of Genomic Lysis Buffer, DNA Stripping Solution and Precipitation Solution proportionally.
  - Introduce optimal Proteinase K step after step 4.
  - 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**
  - Recommend using *MegaLong*™ DNA isolation kit (Cat. # 786-146, 786-147). Isolates nuclei under mild condition, which are then transferred to Tube-O-DIALYZER™, 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.

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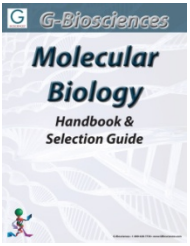

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