G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

OmniPrep™ for Tissue

For High Quality Genomic DNA Extraction From Fresh, Frozen, Fixed & Paraffin-Embedded Tissue, Cells, Non-Mammalian Blood & Gram Negative Bacteria

(Cat. # 786-395)

INTRODUCTION

The *OmniPrep™ for Tissue* kit isolates high quality genomic DNA from tissue samples, including fresh, frozen, fixed or paraffin-embedded tissue. The kit isolates DNA from bodily fluids, including plasma, serum, amniotic fluid, semen and CSF. *OmniPrep™ for Tissue* also isolates high quality DNA from cultured cells, non-mammalian nucleated blood and gram-negative bacteria. The kit isolates high purity (A_{260}/A_{280} ratios of 1.7 to 2) DNA between 100-200kbp and the yield is 0.5-10µg/mg tissue, 30-80µg/ml gram negative bacteria culture and 0.1-40µg/ml body fluid, dependent on starting material and quantity. If used according to the protocols this kit purifies DNA from 2gm solid tissue, $1x10^9$ cultured cells and $1x10^{10}$ gram- negative bacteria.

ITEM(S) SUPPLIED (Cat. # 786-395)

Description	Size
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
Longlife™ Proteinase K *(5mg/ml)*	2 x 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

- Chloroform
- Isopropanol
- 70% Ethanol
- Xylene or safe xylene substitute
- Ethanol for paraffin-embedded tissue

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.

PROTOCOL FOR 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.

FOR 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.

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

PROTOCOL FOR 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, lyze 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 main protocol.

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

PROTOCOL FOR 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 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 1-2 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 main protocol using quantities for 2mg tissue.

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

PROTOCOL FOR 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 main protocol.

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

PROTOCOL FOR 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.*

PROTOCOL FOR NUCLEATED BLOOD CELLS FROM BIRD, FISH & FROG

1. Add 10µl nucleated blood into 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, 1µl nucleated blood is equivalent to 1mg tissue in the table on page2.
2. Continue at step 4 of main protocol.

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

PROTOCOL FOR 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 main protocol.

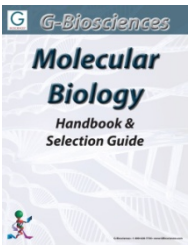
Typical Yield: *30-80µg DNA/1ml Gram negative bacteria.*

CITATIONS

1. Staley, C.A. et al (2012) Gene. 496:118
2. Jonkers, W. et al (2012) Appl Envir Microbiol 78:3656
3. Li, Z. et al (2010) Protein Expression and Purification. 72:113
4. Li, Z. et al (2010) Biochem and Biophys Res Comm. 402:519
5. Lorch, J. et al (2010) J Vet Diagn Invest 22:224
6. Li, Z. et al (2009) Protein Expression and Purification. 67:175
7. Lin-Cereghino, J. et al (2008) Yeast. 25:293
8. Choi, Y. and Shim, W. (2008) Microbiology. 154: 326
9. Choi, Y. et al (2008) Mycologia. 100:701
10. Whitaker, V. et al (2007) J. Amer. Soc. Hort. Sci. 132:534
11. Szabo, L. J. (2007) Mol Ecol Notes 7:92
12. Ordonez, M.E. and Kolmer, J.A. (2007) Phytopathology 97:574
13. Sagaram, U.S. et al (2006) Mol Plant Pathol 7:381
14. Mertens, J.A. et al (2006) Arch. Microbiol. 186:41
15. Lee, B et al (2005) Genome. 48:1104
16. Pliss, L. et al (2004) J. Neurochem. 91:1082
17. Jacobs-Helber, S. et al (2002) JBC. 277:4859
18. Li, X. et al (2002) Genome. 45:229
19. Villar, M. et al (2001) J. Bact. 183:55

RELATED PRODUCTS

Download our Sample Preparation Handbook



http://info.gbiosciences.com/complete-molecular-biology-handbook/

For other related products, visit our website at www.GBiosciences.com or contact us.

Last saved: 9/26/2013 CMH

www.GBiosciences.com