Isolating gDNA from Tissue Samples

Simplified gDNA Isolation from Tissue Samples using the ReliaPrep™ Large Volume HT gDNA Isolation System

Promega Corporation

Sample Type:
Mouse Liver and Mouse Brain

Sample Volume:
5mg–500mg

Yield:

Liver	Brain
5mg = 10µg	25mg = 40µg
10mg = 20µg	100mg = 145µg
25mg = 100µg	250mg = 310µg
50mg = 160µg	
100mg = 320µg	
250mg = 800µg	
500mg = 1,200µg	

Purity:
Liver $A_{260}/A_{280} > 1.87$, $A_{260}/A_{230} = 1.9–2.3$
Brain $A_{260}/A_{280} > 1.85$, $A_{260}/A_{230} = 1.7–2.4$

Size: Greater than 25kb

Eluted Samples:
Ready for downstream assays/archiving

Protocol: *ReliaPrep™ Large Volume HT gDNA Isolation System Technical Manual #*TM341

Disclaimer: This protocol is currently in development at Promega and is based on limited data. We welcome any feedback that may direct ongoing development efforts.

The information provided here is intended for research use applications and not for use in diagnostic procedures.

The ReliaPrep™ Large Volume HT gDNA Isolation System is a scalable, automation-ready system that simplifies gDNA isolation from mouse tissue.

Introduction

This article presents a protocol being developed by Promega R&D Scientists and is not a commercially available method. It is provided for information only and is intended for research applications. It should not be used in diagnostic procedures.

Tissue lysates are created by homogenization, proteinase K digestion and centrifugation of a tissue sample and removal of any remaining solids. Processing with the ReliaPrep™ Large Volume HT gDNA Isolation system uses modified 3ml, 6ml and 10ml ReliaPrep™ protocols for whole blood preprogrammed on the ReliaPrep™ LV 32 HSM Instrument. Tissue lysates are placed into the ReliaPrep™ LV 32 HSM Instrument in 50ml conical tubes for processing. For semi-automated processing, the ReliaPrep™ LV 32 HSM Instrument guides the user through reagent additions and aspirations via its LCD screen based on the specified starting sample volume selected. Launch the ReliaPrep™ method for whole blood, and select the appropriate blood sample volume based on the tissue amount guidelines specified in the protocols below. Some of the initial steps can be ignored by pressing the Enter button and skipped by pressing the down arrow.

Additional Materials Required

Tail Lysis Buffer (TLA), Part# A509X
heater/shaker capable of 55°C overnight incubation

Protocol for up to 25mg of Tissue Samples

All shaking and centrifugation steps in this protocol are at room temperature unless otherwise specified.

1. Add up to 25mg of tissue to 750µl of TLA + 60µl of Proteinase K. Shake samples at 55°C overnight.
2. Optional: Any remaining tissue aggregates should be removed via centrifugation.
3. Place samples on the HSM instrument, and select the 3ml blood protocol by pressing enter and ignoring the prompts for addition of Proteinase K and Alkaline Protease. Advance the HSM instrument to the addition of Lysis Buffer.
4. Add 3ml of Lysis Buffer to each sample by pressing the down arrow on the instrument once the heated lysis shake begins and again once the nonheated lysis shake begins. Advance the HSM instrument to the addition of Binding Buffer.
5. Add 3.6ml of Binding Buffer to each sample.

6. Thoroughly resuspend ReliaPrep™ Resin, and add 300µl of the resin to each sample. Bind the nucleic acids to the resin by shaking at 500rpm for 20 minutes. Collect the resin for 14 minutes using a magnet.
7. Remove waste from the binding step from each tube by aspirating or pipetting. After removing waste, add 1ml of 50% Ethanol Wash and 4ml of Wash Buffer to each tube.
8. Shake samples at 600rpm for 2 minutes.
9. After shaking, thoroughly mix the samples 5 times using the tips. Shake at 600rpm for 2 minutes in the HSM instrument. Capture the resin for 3 minutes using the magnet.
10. Remove waste from the first wash step from each tube by aspirating or pipetting. After removing waste, add 1ml of 50% Ethanol Wash and 4ml of Wash Buffer to each tube.
11. Shake samples at 600rpm for 4 minutes, and capture the resin on the magnet for 3 minutes.
12. Remove waste from the second wash step from each tube by aspirating or pipetting. After removing waste, add 4ml of 50% Ethanol Wash to each tube.
13. Shake samples at 550rpm for 4 minutes, and capture the resin on the magnet for 3 minutes.
14. Add elution buffer (1.5ml of Nuclease-Free Water) to each tube. Shake samples at 600rpm for 3 minutes, then at 400rpm for 15 minutes at 80°C. Capture the resin for 5 minutes using a magnet.
15. Transfer 1ml of the eluate to the intermediate labware.
16. Centrifuge the intermediate labware at 2,500 × *g* for 10 minutes to remove any particulates.
17. Transfer the eluates to the final elution labware.
18. The method is finished.

Protocol for 25–100mg of Tissue Samples

All shaking and centrifugation steps in this protocol are at room temperature unless otherwise specified.

1. Add 25–100mg of tissue to 1.5ml of TLA + 120µl of Proteinase K Solution. Shake samples at 55°C overnight.
2. Optional: Any remaining tissue aggregates should be removed via centrifugation.
3. Place samples on the HSM instrument, and select the 6ml blood protocol by pressing enter and ignoring the prompts for addition of Proteinase K and Alkaline Protease. Advance the HSM instrument to the addition of Lysis Buffer.
4. Add 6ml of Lysis Buffer to each sample by pressing the down arrow on the instrument once the heated lysis shake begins and again once the nonheated lysis shake begins. Advance the HSM instrument to the addition of Binding Buffer.
5. Add 7.2ml of Binding Buffer to each sample.
6. Thoroughly resuspend ReliaPrep™ Resin, and add 600µl of the resin to each sample. Bind the nucleic acids to the resin by shaking at 500rpm for 20 minutes. Collect the resin for 14 minutes using a magnet.
7. Remove waste from the binding step from each tube by aspirating or pipetting. After removing waste, add 1ml of 50% Ethanol Wash and 6ml of Wash Buffer to each tube.
8. Shake samples at 600rpm for 2 minutes.
9. After shaking, thoroughly mix the samples 5 times using the tips. Shake at 600rpm for 2 minutes in the HSM instrument. Capture the resin for 3 minutes using the magnet.
10. Remove waste from the first wash step from each tube by aspirating or pipetting. After removing waste, add 1ml of 50% Ethanol Wash and 6ml of Wash Buffer to each tube.
11. Shake samples at 600rpm for 4 minutes, and capture the resin on the magnet for 3 minutes.
12. Remove waste from the second wash step from each tube by aspirating or pipetting. After removing waste, add 6ml of 50% Ethanol Wash to each tube.
13. Shake samples at 550rpm for 4 minutes, and capture the resin on the magnet for 3 minutes.
14. Add elution buffer (1.5ml of Nuclease-Free Water) to each tube. Shake samples at 600rpm for 3 minutes, then at 400rpm for 15 minutes at 80°C. Capture the resin for 5 minutes using a magnet.
15. Transfer 1ml of the eluate to the intermediate labware.

16. Centrifuge the intermediate labware at 2,500 × *g* for 10 minutes to remove any particulates.
17. Transfer the eluates to the final elution labware.
18. The method is finished.

Protocol for 250–500mg of Tissue Samples

All shaking and centrifugation steps in this protocol are at room temperature unless otherwise specified.

1. Add 250–500mg of tissue to 2.5ml of TLA + 225µl of Proteinase K Solution. Shake samples at 55°C overnight.
2. Optional: Any remaining tissue aggregates should be removed via centrifugation.
3. Place samples on the HSM instrument, and select the 10ml blood protocol by pressing enter and ignoring the prompts for addition of Proteinase K and Alkaline Protease. Advance the HSM instrument to the addition of Lysis Buffer.
4. Add 10ml of Lysis Buffer to each sample by pressing the down arrow on the instrument once the heated lysis shake begins and again once the nonheated lysis shake begins. Advance the HSM instrument to the addition of Binding Buffer.
5. Add 12ml of Binding Buffer to each sample.
6. Thoroughly resuspend ReliaPrep™ Resin, and add 1ml of the resin to each sample. Bind the nucleic acids to the resin by shaking at 500rpm for 20 minutes. Collect the resin for 14 minutes using a magnet.
7. Remove waste from the binding from each tube by aspirating or pipetting. After removing waste, add 1ml of 50% Ethanol Wash and 8ml of Wash Buffer to each tube.
8. Shake samples at 600rpm for 2 minutes.
9. After shaking, thoroughly mix the samples 5 times using the tips. Shake at 600rpm for 2 minutes in the HSM instrument. Capture the resin for 3 minutes using the magnet.
10. Remove waste from the first wash step from each tube by aspirating or pipetting. After removing waste, add 1ml of 50% Ethanol Wash and 8ml of Wash Buffer to each tube.
11. Shake samples at 600rpm for 4 minutes, and capture the resin on the magnet for 3 minutes.
12. Remove waste from the second wash step from each tube by aspirating or pipetting. After removing waste, add 8ml of 50% Ethanol Wash to each tube.
13. Shake samples at 550rpm for 4 minutes, and capture the resin on the magnet for 3 minutes.
14. Add elution buffer (1.5ml of Nuclease-Free Water) to each tube. Shake samples at 600rpm for 3 minutes, then at 400rpm for 15 minutes at 80°C. Capture the resin for 5 minutes using a magnet.
15. Transfer 1ml of the eluate to the intermediate labware.
16. Centrifuge the intermediate labware at 2,500 × *g* for 10 minutes to remove any particulates.
17. Transfer the eluates to the final elution labware.
18. The method is finished.

Ordering Information

Product	Size	Cat.#
ReliaPrep™ Large Volume HT gDNA Isolation System	96 × 10ml or 960 × 1ml preps	A1751
ReliaPrep™ LV 32 HSM Instrument	1 each	A1715