Isolating gDNA from Urine Samples

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

Promega Corporation

Sample Type:
Unfiltered Human Urine—Fresh

Sample Volume:
50ml

Yield: 600ng to over 1,000ng of DNA from 50ml samples.
Yield of gDNA will vary greatly depending on the individual donating the sample.

Purity: A_{260}/A_{280} = N/A
A_{260}/A_{230} = N/A

Size: N/A

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 human urine.

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.

This method has only been tested with human urine samples. Samples from other species have not been tested.

Urine cell pellets are created by centrifugation of a urine sample and removal of the liquid fraction. Processing with the ReliaPrep™ Large Volume HT gDNA Isolation System is based on using the ReliaPrep™ method for a 10ml whole blood sample. Urine cell pellet samples 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 ReliaPrep™ 10ml whole blood sample method.

Optional Materials Required

RNase (e.g., RNase A Solution, Cat.# A7974)

Protocol for 50ml of Urine Samples

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

1. Add 50ml of unfiltered human urine to a 50ml conical tube and centrifuge at 2,000 × *g* for 10 minutes to pellet the cells. Carefully pour off the supernatant, and place the tube with the pellet in the HSM instrument.
2. Optional: Add 80µl of RNase to each sample. This volume is not indicated in the semi-automated protocol.
3. Add 200µl of Proteinase K Solution to each sample. Shake samples at 500rpm for 1 minute.
4. Add 1.25ml of Alkaline Protease to each sample. Shake samples at 500rpm for 1 minute.
5. Add 10ml of Lysis Buffer to each sample. Shake samples at 500rpm for 20 minutes at 65°C, then at 500rpm for 20 minutes at room temperature.
6. Add 12ml of Binding Buffer to each sample.

7. 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.
8. Remove waste from the binding 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.
9. Shake samples at 600rpm for 2 minutes.
10. 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.
11. 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.
12. Shake samples at 600rpm for 4 minutes, and capture the resin on the magnet for 3 minutes.
13. 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. 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 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