Presto™ Urine DNA Extraction Kit

CERTIFICATE NO. QAIC/TW/50077
ISO 9001:2015 QMS

For research use only

Catalogue Numbers	Quantity
UR002	25 rxns
UR050	50 rxns
UR100	100 rxns

Introduction

The Presto™ Urine DNA Extraction Kit is designed for rapid isolation of high-quality cellular and cell-free DNA from up to 5 ml of urine. The kit includes uniquely designed Column Extension Tubes which allow for increased sample volume and yield. Urine samples are lysed using Proteinase K and a buffer containing chaotropic salt. The lysate is mixed with a binding buffer to facilitate DNA binding to the column membrane. The column is then washed and DNA is eluted with Elution Buffer. The entire procedure can be completed within 60 minutes and the purified DNA is ready for use in a variety of downstream applications such as qPCR, Next-Generation sequencing and DNA methylation analysis.

Quality Control

The quality of the Presto™ Urine Extraction Kit is tested on a lot-to-lot basis by isolating DNA from 2 ml of urine. Following the purification process, the purified DNA integrity was assessed by qPCR.

Specifications

- Purify cellular and cell-free DNA within 60 minutes
- Sample: 1-5 ml of urine
- Format: Spin columns combined with column extension tubes using vacuum or centrifuge
- Yield: 1-20 ng of DNA per ml of male urine. 5-1000 ng of DNA per ml of female urine.
- Elution Volume: 30-50 µl
- Applications: qPCR, Next-Generation sequencing and DNA methylation analysis
- Storage: dry at room temperature (20~25ºC)

Caution

During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.

Components

Product Name	UR002	UR050	UR100
UR1 Buffer	1.5 ml	30 ml	60 ml
UR2 Buffer	12 ml	275 ml	275 ml X2
Proteinase K[1] (add ddH_2O)	5 mg (0.5 ml)	65 mg (6.5 ml)	65 mg x2 (6.5 ml x2)
W1 Buffer	2 ml	45 ml	45 ml
Wash Buffer[2] (Add Ethanol)	1 ml (4 ml)	12.5 ml (50 ml)	25 ml (100 ml)
Elution Buffer	1 ml x2	6 ml	10 ml
Carrier RNA[3] (add Elution Buffer)	1 mg (1 ml)	1 mg (1 ml)	1 mg (1 ml)
Column Extender	2 pcs	50 pcs	100 pcs
GD Column	2 pcs	50 pcs	100 pcs
2 ml Collection Tube	2 pcs	50 pcs	100 pcs

Product	Package Size	Catalogue Number
Genomic DNA Mini kit (blood/Cultured Cell)	100/300 preps	GB100/300
Genomic DNA Maxi kit (blood/Cultured Cell)	10/25 preps	GDM010/25
Genomic DNA Mini Kit (Tissue)	50/100/300 perps	GT050/100/300
gSYNC™ DNA Extraction Kit	50/100/300 perps	GS050/100/300
Genomic DNA Mini Kit (Plant)	100 preps	GP100
Genomic DNA Maxi kit (Plant)	10/25 preps	GPM010/25
GENEzol™ DNA Reagent Plant	100/200 runs	GR100/200
Presto™ Mini gDNA Yeast Kit	100/300 preps	GBY100/300
Presto™ Mini gDNA Bacteria Kit	100/300 preps	GBB100/101/300/301
Geneius™ Micro DNA Extraction Kit	100/300 preps	GMB100/300
Presto™ Buccal Swab gDNA Extraction Kit	100/300 preps	GSK100/300
Presto™ 96 Well Blood gDNA Extraction Kit	4/10 x 96 preps	96GBP04/10
Presto™ 96 Well Plant gDNA Extraction Kit	4/10 x 96 preps	96GPP04/10

[1] Add ddH_2O to Proteinase K (see the bottle label for volume) then vortex to ensure it is completely dissolved. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. For extended periods, the ddH_2O and Proteinase K mixture should be stored at 4ºC.

[2] Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

[3] Add 1 ml of Elution Buffer to Carrier RNA then vortex to ensure it is completely dissolved to obtain a working solution of 1 µg/µl. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. Divide the Carrier RNA solution into convenient volumes in several RNase-free 1.5 ml microcentrifuge tubes. The Carrier RNA solution should be stored at -20ºC. Do not freeze and thaw the Carrier RNA solution more than 3 times.

Additional Requirements

1.5 ml microcentrifuge tubes, 50 ml centrifuge tubes, 60°C water bath or dry bath, absolute ethanol, isopropanol. Vacuum manifold for vacuum protocol and centrifuge with 50 ml centrifuge tube swing bucket for centrifuge protocol.

Presto™ Urine DNA Extraction Kit Procedure

Please read the entire instruction manual prior to starting the Protocol Procedure.

Sample preparation

For extracting cell-free DNA only from urine

- Transfer up to 5 ml of urine into a 15 ml centrifuge tube, centrifuge at 3,000 xg for 15 minutes.
- Carefully transfer the urine supernatant into a clean 15 ml/50 ml centrifuge tube without disturbing the pellet.
- proceed with step 1 sample lysis.

For extracting total DNA from urine

Proceed with step 1 sample lysis.

Step 1 Sample Lysis

- UR1 Buffer preparation: Add **1 µl of Carrier RNA** solution to appropriate volume of **UR1 Buffer** (see the table below) and vortex shortly to mix.

Urine volume	1 ml	2 ml	3 ml	4 ml	5 ml
UR1 Buffer	100 µl	200 µl	300 µl	400 µl	500 µl
Carrier RNA	1 µl	1 µl	1 µl	1 µl	1 µl

- Add the **Proteinase K, urine sample** and **UR1 Buffer containing carrier RNA** to a clean 15 ml/50 ml centrifuge tube (see the table below). Close the cap and mix by vortex for 30 seconds.

Urine volume	1 ml	2 ml	3 ml	4 ml	5 ml
Proteinase K	25 µl	50 µl	75 µl	100 µl	125 µl
UR1 Buffer containing carrier RNA	100 µl	200 µl	300 µl	400 µl	500 µl
Centrifuge tube size	15 ml	15 ml	15 ml	50 ml	50 ml

- Incubate in a 60°C water bath or dry bath for 30 minutes.
  Note: At this time, preheat the required Elution Buffer (50 µl per sample) to 60ºC (for Step 4 DNA Elution).

- Add appropriate volume of **UR2 Buffer** and absolute ethanol into the sample lysate (see the table below). Close the cap and mix by vortex for 10 seconds.

Urine volume	1 ml	2 ml	3 ml	4 ml	5 ml
UR2 Buffer	1 ml	2 ml	3 ml	4 ml	5 ml
Absolute ethanol	1 ml	2 ml	3 ml	4 ml	5 ml

Step 2 DNA Binding

Centrifuge Protocol

- Connect the **GD column** with the **Column Extender** and place the GD column assembly into a clean 50 ml centrifuge tube.
- Transfer 10 ml of the sample mixture into the GD column assembly and centrifuge at 1,500 x g for 2 minutes. Discard the flow-through and repeat until the entire sample mixture has passed through the GD column.
- Disconnect the GD column from the column extender and place the GD column in a **2 ml Collection Tube**.

Vacuum Protocol

- Connect the **GD column** with the **Column Extender** and place the GD column assembly onto a vacuum manifold.
- Transfer the entire sample mixture into the GD column assembly. Apply vacuum at 15 inches Hg until sample passes through the GD column assembly, switch off the vacuum.
- Disconnect the GD column from the column extender and place the GD column in a **2 ml Collection Tube**.

Step 3 Wash

- Add **400 µl of W1 Buffer** to the **GD Column**. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the **GD Column** back in the 2 ml Collection Tube.
- Add **600 µl of Wash Buffer (make sure absolute ethanol was added)** to the **GD Column**. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the **GD Column** back in the 2 ml Collection Tube.
- Wash the **GD column** with **600 µl of Wash Buffer (make sure absolute ethanol was added)** again. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the **GD Column** back in the 2 ml Collection Tube.
- Centrifuge at 16,000 x g for 3 minutes at room temperature to dry the column matrix.

Step 4 DNA Elution

- Transfer the dried **GD Colum**n to a new 1.5 ml microcentrifuge tube.
- Add **30-50 µl of pre-heated Elution Buffer**[1] into the CENTER of the column matrix. Let stand for at least 2 minutes to allow Elution Buffer to be completely absorbed.
- Centrifuge at 16,000 x g for 2 minutes at room temperature to elute the purified DNA. Eluted DNA can be used immediately for downstream applications or stored at -20°C.

Note:

[1] If a higher DNA concentration is required, use 30 µl of Elution Buffer then repeat the Elution step by adding the same 30 µl of Elution Buffer (which now contains the eluted DNA) to the center of the column matrix again. If maximum DNA yield is required, use 50 µl of Elution Buffer (DNA concentration will be diluted). Ensure that Elution Buffer is added into the center of the GD Column matrix and is completely absorbed.

Troubleshooting

Problem	Cause	Solution
Low nucleic acid yield	A. Incomplete sample lysis B. Inappropriate buffer preparation	A. Ensure adding appropriate volume of UR1 Buffer and Proteinase K into urine sample and digestions are performed at 60 ˚C. It is possible to extend digestion times to 1 hour if samples are high in protein. B. Add appropriate volume of absolute ethanol (see the bottle label) to the Wash Buffer prior to use.
Eluted DNA does not perform well in downstream applications	A. Residual ethanol contamination B. Interference due to carrier RNA	A. Following the wash step, dry the GD Column with additional centrifugation at 16,000 x g for 3 minutes to remove residual ethanol. B. If the presence of carrier RNA in the eluate interferes with the downstream enzymatic reaction, it may be necessary to reduce the amount of carrier RNA or to omit it altogether.