i-genomic Urine DNA Extraction Mini Kit

The Instruction Manual for Genomic DNA Extraction from urine samples using silica membrane.

RUO *Research Use Only* REF *17391* Σ 50

DESCRIPTION

- Urine is the low concentration of nucleated cells present in human urine. The nucleated cells found in urine are typically white blood cells and epithelial cells. There are a number of advantages to using the DNA found in urine for diagnostics, including the fact that sample collection of urine is absolutely non-invasive, and the isolation of DNA from urine is technically easier than from blood due to the low protein content of urine.
- Furthermore, urine is non-infectious for HIV and less infectious for many other pathogens. However, the current methods for isolating DNA from urine have a number of drawbacks associated with them. First of all, the methods are generally long and tedious, and only result in trace amounts of DNA being isolated. Furthermore, large amounts of urine are required for the procedure, however only very small amounts of DNA are isolated. Also, the procedures often involve the use of expensive or harmful chemicals.
- i-genomic Urine DNA Extraction Mini Kit provide a fast and simple protocol for isolation. The purified DNA is free of contaminants and impurities and is ideal for all PCR, southern blotting, RAPD, and sequencing applications. i-genomic Urine DNA Extraction Mini Kit uses advanced silica-gel membrane technology for rapid and efficient purification of genomic DNA without organic extraction or ethanol precipitation. Furthermore, Buffer UG system is optimized to allow rapid and simple cell lysis followed by selective binding of DNA to the column.

CHARACTERISTICS

The Best Way to GENOMIC DNA

- **Speed** : Takes only 20 ~ 30 minutes to extract genomic DNA.
- **Smart** : Ideal for cytosolic (pathogen) DNA extraction. High quality and quantity of DNA recovery
- **Steady** : Complete removal of inhibitors and contaminants for accurate down stream applications. And the freeze-dried formulated enzyme has been improved DNA extraction stability.
- **Stage-up** : No need various DNA Extraction kit – vast applicability. The Kit is suitable to use various kinds of biological samples. Advanced GxN technology for rapid and efficient purification of DNA without ethanol precipitation.

KIT CONTENTS

Label	Description	Contain
Buffer UG	Lysis Buffer	12 ml
Buffer UB	Binding Buffer	14 ml
Buffer UWA (concentrate) [1]	Washing Buffer A	12 ml
Buffer UWB (concentrate) [2]	Washing Buffer B	10 ml
Buffer UE[3]	Elution Buffer	20 ml
Spin Columns (Violet O-ring Color)	Inserted into a Collection Tubes. (2.0 ml tubes)	50 columns
Collection Tubes (2.0ml tubes)	Additionally supplied.	100 tubes
RNase A[4] **(Lyophilized powder)**	Dissolve with Pure DW 0.3 ml	3 mg
Proteinase K[5] **(Lyophilized powder)**	Dissolve with Pure DW 1.1 ml	22 mg

1. Buffer UWA is supplied as concentrates. Add 28 ml of ethanol (96~100%) according to the bottle label before use.
2. Buffer UWB is supplied as concentrates. Add 40 ml of ethanol (96~100%) according to the bottle label before use.
3. Buffer UE are finally 10mM Tris-HCl (pH 8.0). You may use your lab's buffer.
4. Lyophilized RNase A : Dissolve the RNase A in 0.3 ml of pure D.W. to each vial. The lyophilized RNase A can be stored at room temperature (15–25°C) until the expiration date without affecting performance. The lyophilized RNase A can only be dissolved in D.W.; dissolved RNase A should be immediately stored at -20℃. The RNase A solution is stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date.
5. Lyophilized Proteinase K : Dissolve the Proteinase K in 1.1 ml of pure D.W. to each vial. The lyophilized Proteinase K can be stored at room temperature (15–25°C) until the expiration date without affecting performance. The lyophilized Proteinase K can only be dissolved in D.W.; dissolved Proteinase K should be immediately stored at -20℃. The Proteinase K solution is stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date.

STORAGE

i-genomic Urine DNA Extraction Mini Kit should be stored dry at room temperature (15–25°C). Under these conditions, i-genomic Urine DNA Extraction Mini Kit can be stored for up to 24 months without showing any reduction in performance and quality. The lyophilized RNase A and Proteinase K can be stored at room temperature (15–25°C) until the kit expiration date without affecting performance. The lyophilized enzyme can only be dissolved in D.W.; dissolved enzyme should be immediately stored at -20℃. These solutions are stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date.

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

All products undergo extensive quality control test and are warranted to perform as described when used correctly. Immediately any problems should be reported. Satisfaction guarantee is conditional upon the customer providing full details of the problem to iNtRON within 60 days, and returning the product to iNtRON for examination.

PRODUCT USE LIMITATIONS

All i-genomic series Kits are developed, designed, and sold for research purpose only. They are not to be used for human or animal diagnosis of diseases. Do not use internally or externally in humans or animals. Be careful in the handling of the products.

NOTICE BEFORE USE

i-genomic Urine DNA Extraction Mini Kit provides almost all reagents for extracting DNA, including lyophilized enzyme. However, you should prepare some equipments and reagents as follows for a fast and easy extraction. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

• Common equipment and reagents

- Equipment for disruption and homogenization, including Grinding Jar Set (mortar)
- Pipettes and pipette tips
- Vortex mixer
- 80% EtOH
- Microcentrifuge with rotor for 2.0 ml tubes
- Ice
- Lyticase or zymolase solution
- Water bath or heating block
- Absolute ethanol (EtOH, 96~100%)
- Liquid nitrogen
- Microcentrifuge tubes (1.5 ml)
- 15ml tube
- Other general lab equipments

SAFETY INFORMATION

All chemicals should be considered as potentially hazardous. When working with chemicals, always should wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please request the appropriate material safety data sheets (MSDS). Do not add bleach or acidic solutions directly to the waste.

CAUTION:
DO NOT add bleach or acidic solutions directly to the sample preparation waste.

APPLICATIONS

- Bacterial genomic research
- Pathogen detection study
- Detection Assay : PCR, real time PCR

QUALITY CONTROL

- In accordance with iNtRON's ISO-certified Total Quality Management System, each lot of i-genomic Urine DNA Extraction Mini Kit is tested against predetermined specifications to ensure consistent product quality. The quality of the isolated genomic DNA was checked by restriction analysis, agarose gel electrophoresis, and spectrophotometric determination.
- i-genomic spin column control : The DNA binding capacity was tested by determining the recovery with 10 ~ 15 μg of genomic DNA from 1 x 10^6 cells.
- RNase A / Proteinase K : In case of RNase A, the activity was determined 20K ~ 25K unit per mg of protein using tolura yeast RNA hydration test. In case of Proteinase K, the activity was determined from cleavage of the substrate releasing p-nitroaniline which can be measured spectrophotometrically at 410nm.
- Buffer control : Conductivity and pH of buffers were tested and found to be within the pre-determinated ranges descrbed below

Table 1. Quality control criteria of each components

Buffer	Conductivity	pH
Buffer UG	14.5 ~ 16.5 mS/cm	8.3 ~ 8.6
Buffer UB	95 ~ 105 mS/cm	7.4 ~ 7.9
Buffer UWA	45 ~ 50 mS/cm	4.5 ~ 4.9
Buffer UWB	10 ~ 12 mS/cm	7.5 ~ 8.0
Buffer UE	500 ~ 700 μS/cm	7.2 ~ 8.1

COLUMN INFORMATION

Table 3. Column informations of i-genomic Series

Column Membrane [1]	Silica-based membrane
Spin Column [1]	Individually, is inserted in a 2.0 ml collection tube [2].
Loading Capacity	Maximum 800 μl
DNA Binding Capacity	Maximum 45 μg
Recovery	85 ~ 95% depending on the elution volume
Elution Volume	Generally, eluted with 30 ~ 200 μl of Elution Buffer

1. After use, seal the pack containing spin columns tightly without getting dry. Then, the spin columns are stable for over 2 years under these conditions. It's not good for DNA binding to be dried completely.
2. Additional collection tubes (100 ea) are also supplied for your convenient handling.

IMPORTANT POINTS BEFORE STARTING

- **Buffer UWA / UWB :** Buffer UWA / UWB is supplied as concentrate. Before using for the first time, be sure to add 28 ml / 40 ml of absolute ethanol (96 ~ 100%) to obtain a working solution.
- **Lyophilized enzyme** Dissolve the lyophilized enzyme in appropriate volume of pure D.W. - Lyophilized RNase A : Dissolve the RNase A in 0.3 ml of pure D.W. - Lyophilized Proteinase K : Dissolve the Proteinase K in 1.1 ml of pure D.W. The lyophilized enzyme can be stored at room temperature (15–25°C) until the expiration date without affecting performance. The lyophilized enzyme can only be dissolved in D.W.; dissolved enzyme should be immediately stored at -20℃. The enzyme solution is stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date
- **Preheat a water bath or heating Block**
- **Centrifugation :** All centrifugation steps are carried out at RT (15 ~ 25℃) in a micro-centrifuge.

PROTOCOL

: Refer to the "VISUAL PROTOCOL"

1. **Collect urine sample into a sterile 50 ml tube. Then concentrate the sample to final volume of 100 μl by centrifugation.**
   Note : Use centrifugal microcon-centrator such as Centricon-100, Microseq-100 or equivalent from other suppliers.
   Note : Some samples, plasma in particular, may be difficult to concentrate to 100 μl due to high viscosity. Centrifugation for up to 6 hours may be necessary.
2. **Add 200 μl of Buffer UG, 20 μl of Proteinase K Solution, and 5 μl of RNase A Solution into sample tube, and mix by vortex vigorously.**
3. **Incubate the lysate for 15 min at 65 ℃.**
   Note : For complete lysis, mix 5 ~ 6 times during incubation by inverting tube.
4. **After lysis completely, add 250 μl of Buffer UB to the lysate, and mix by pipetting or gently inverting 5 to 6 times. DO NOT vortex. After mixing, spin down to remove drops from inside the lid.**
5. **Add 800 μl of 80% ethanol to the lysate, and mix by pipetting or gently inverting 5 to 6 times. DO NOT vortex. After mixing, spin down to remove drops from inside the lid.**

   Note : It is essential that the sample and 80% ethanol are mixed thoroughly to yield a homogeneous solution. But do NOT vortex vigorously, because high speed of vortexing can give occasion to shearing of genomic DNA.
6. **Pipette 800 μl of the mixture from step 5 into the spin column inserted in a 2.0 ml collection tube. Centrifuge for 1 min at 13,000 rpm (RT), and discard the flow-through and collection tube altogether.**
   Note : The maximum volume of the spin column reservoirs is 800 μl.
7. **Place the spin column into a new 2.0 ml collection tube(additionally supplied), add 700 μl of Buffer UWA to the spin column, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and reuse the collection tube in step 8.**
   Note : Ensure that 28 ml of ethanol (EtOH) has been added to Buffer UWA.
8. **Add 700 μl of Buffer UWB to column and centrifuge for 1 min at 13,000 rpm.**
   Note : Ensure that 40 ml of ethanol (EtOH) has been added to Buffer UWB.
9. **Discard the flow-through and again centrifuge for additionally 1 min to dry the membrane. Discard the flow-through and collection tube altogether.**
   Note : It is very important to dry the membrane of the spin column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the spin column from the collection tube without contacting with the flow-through, since this will result in carryover of ethanol.
10. **Place the spin column into a new 1.5 ml tube (not supplied), and 50 μl of Buffer UE directly onto the membrane. Incubate for 1 min at room temperature, and then centrifuge for 1 min at 13,000 rpm to elute.** **Note** : Elution with 50 μl increases the final DNA concentration, but reduces overall DNA yield conventionally. Alternatively, if you need larger amounts of DNA, eluting with 100 μl increases generally overall DNA yield.

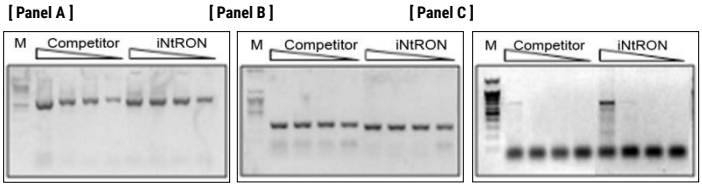
TROUBLESHOOTING GUIDE

When working with i-genomic Urine DNA Extraction Mini Kit, always follow the description of each protocols. Nevertheless, if it causes problems upon extracting DNA, please refer to the following Troubleshooting Guide. This Troubleshooting Guide may be helpful in solving any problems that may arise. For more information, please contact our Technical Assist Team. Our Technical Assist Teams are staffed by experienced researchers with extensive practical and theoretical expertise in molecular biology and the use of iNtRON products.

Problem	Possible Cause	Recommendation
Low DNA yield	Inadequate lysis	▪ Reduce the amounts of starting material.
		▪ Increase the incubation time at 65°C in Lysis step.
	Error in DNA binding	▪ After adding ethanol in DNA Binding step, please mix well by gently inverting.
		▪ Check that the amount of ethanol is added correctly to the supernatant.
	Incorrect Washing step	▪ Check again that the amount of ethanol is added correctly to Buffer UWA and UWB.
		▪ When store Buffer UWA and UWB, always keep a lid shut tightly without evaporation.
	Insufficient DNA elution	▪ Increase the volume of Buffer UE or water to 100 µl
		▪ Increase the incubation time on the column to 5 ~ 10 min at room temperature prior to centrifugation.
		▪ Reload the elute, and then incubation for 1 min at room temperature prior to centrifugation
Low flow rate in column	Clogged Spin Column by particulate material	▪ Increase the incubation time at 65°C in Lysis step.
	High viscosity of Lysate Reagents correctly	▪ Reduce the amounts of starting material.
		▪ Wash the pellet with PBS
		▪ Increase the incubation time at 65°C in Lysis step.
	Problem in centrifugation	▪ Check your centrifuge, and then speed up or increase the centrifugation time.
DNA sheared	Incorrect storage of sample	▪ When storing frozen sample, always keep the samples frozen below -70°C.
		▪ If possible, it is preferable to use fresh tissues.
	Vigorously vortex	▪ Do not vortex the mixture after adding ethanol as described in protocol.
Problems in downstream experiments	Ethanol contamination	▪ Ensure that during Washing Step B, the column membrane should be dried completely. Please centrifuge at full speed for 5 ~ 10 min to dry the membrane.
		▪ During Washing Step B, after centrifugation, remove carefully the Spin Column from the collection tubes without contacting with the flow-through. This careless contact will result in contamination of ethanol.
	Salt contamination	▪ Wash again the Spin Column with Buffer UWB
		▪ Store Buffer UWA and UWB at room temperature (15 ~ 20°C).
	Amount of DNA used in experiments.	▪ Optimize the amount of DNA used in your downstream experiments.

EXPERIMENTAL INFORMATION

Microbial DNA Extraction efficiency from Urine Sample



[Panel A] Gram-Positive Bacteria; The PCR results of *B. subtilis*
The bacterial 16S rDNA (app 1.1 Kb) was amplified with purified DNA from filtered urine and Bacterial mixture.
The band intensity of iNtRON's amplicon is better than competitor's.

[Panel B] Mycoplasma; The PCR results of *M. fermentas*
The Mycoplasma specific gene (app 270 bp) was amplified with purified DNA from Filtered urine and Mycoplasma mixture.
The band intensity of iNtRON's amplicon is similar with competitor's.

[Panel C] Yeast; The PCR results of *C. albicans*
The yeast specific 18S rDNA gene (app 730 bp) was amplified with purified DNA from filtered urine and *C. albicans* mixture.
The band intensity of iNtRON's amplicon is similar with competitor's.

RELATED PRODUCT

Product Name	Cat. No.
Maxime™ PCR PreMix (*i*-Taq)	25025 / 25026
Maxime™ PCR PreMix (*i*-StarTaq)	25165 / 25167
Maxime™ PCR PreMix (*i*-pfu)	25185
Maxime™ PCR PreMix (*i*-MAXII)	25265
PCR Starter Kit (MS-1 Type)	25141
PCR Starter Kit (MS-2 Type)	25142
Agarose	32032
RedSafe™ Nucleic Acid Staining Solution	21141
SiZer™-1000 DNA Marker Solution	24074
SiZer™-100 DNA Marker Solution	24073
G-spin™ Total DNA Extraction Mini Kit	17045
i-genomic Clinic DNA Extraction Mini Kit	17384
i-genomic Stool DNA Extraction Mini Kit	17451

VISUAL PROTOCOL

I Preparation step

1

Harvest the Urine Sample

Centrifuge 4,000 rpm for 15 min

Discard the supernatant and Resuspend the pellet with app 100㎕ of remnant

Transfer the suspension into a new 1.5ml microtube

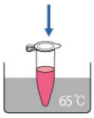
II. Lysis step

2

Add 200㎕ of Buffer UG
20㎕ of Proteinase K
5㎕ of RNase A

Mix by vortexing vigorously (or mix by pipetting)

3



Incubate at 65℃ for 15 mins (invert the tube every 2~3 min)

III Binding step

4

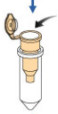
Add 250㎕ of Buffer UB and
mix by pipetting or gently vortexing for 5~6 times
Do Not Vertex

5

Add 250㎕ of 80% EtOH and mix by pipetting or gently vortexing for 5~6 times
Do Not Vertex

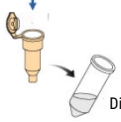
Spin down

6



Transfer the mixture to Spin Column

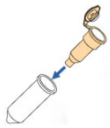
Centrifuge 13,000 rpm for 1 min



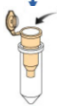
Discard

IV Washing step

7

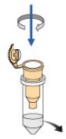


Place Spin Column into a new 2.0 ml Collection Tube



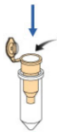
Add 700㎕ of Buffer UWA

Note :
Ensure that 28 ml of EtOH has been added to buffer UWA



Centrifuge 13,000 rpm for 1 min

Discard the flow-through



Add 700㎕ of Buffer UWB

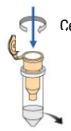
8

Note :
Ensure that 40 ml of EtOH has been added to buffer UWB

Centrifuge 13,000 rpm for 1 min

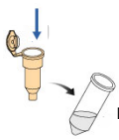
9

Discard the flow-through



Centrifuge 13,000 rpm for 1 min

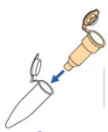
Discard the flow-through



Discard

V. Elution step

10

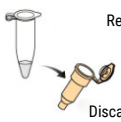


Transfer Spin Column to new 1.5ml Tube

Add 50㎕ of Buffer UE directly onto the membrane

Incubate at RT for 1 min

Centrifuge 13,000 rpm for 1 min



Recover the final eluate (gDNA)

Discard