i-genomic Stool plus DNA Extraction Mini Kit

For Genomic DNA Mini prep. in stool sample

RUO *Research Use Only* REF 17452 Σ 50 15℃ 25℃

DESCRIPTION

i-genomic Stool plus DNA Extraction Mini Kit is based on silica membrane technology and provides special buffer system with InhibitEX Tablet for stool sample gDNA extraction. The spin column is new typed-silica membrane which can bind DNA optimally on given salt and pH conditions. Stool samples typically contain many compounds that can degrade DNA and inhibit downstream enzymatic reactions. To ensure removal of these substances, the i-genomic Stool plus DNA Extraction Mini Kit contains InhibitEX Tablet, a special tool provided in a convenient tablet form. The newly added InhibitEX Tablet can absorb impurity from sample easily. It is possible to completely remove impurity, proteins and other organic compound through the simple centrifugation processing. The genomic DNA isolated with these products guarantees high quality, high purity and full-length. Purified genomic DNA is ready for use in downstream applications such as PCR.

KIT CONTENTS

Component	50 prep	Storage
SL Buffer*	80 ml	
SB Buffer**	15 ml	
SW1 Buffer[1]	13 ml	
SW2 Buffer[2]	15 ml	
SE Buffer	15 ml	15℃ to 25℃
Proteinase K	1 ml	
InhibitEX Tablets	50 ea	
Spin Columns	50 ea	
Collection Tubes	50 ea	

*/** If precipitates formed in SL Buffer or SB Buffer, warm buffer to 37℃ until precipitates fully dissolve.

1/2 Ensure that SW1 Buffer and SW2 Buffer have been prepared with appropriate volume of ethanol (96 ~ 100%) as indicated on the bottle and shake thoroughly before use.

STORAGE

i-genomic Stool plus DNA Extraction Mini Kit can be stored in dry condition and room temperature (15 - 25℃) for up to 12 months without resulting of any reduction in performance and quality. For longer storage, the kit can be stored at 2 - 8℃. If a precipitate has formed in buffer under 2 - 8℃, please place the buffer at room temperature or warm at 37℃ for 10min to dissolve the precipitate.

IMPORTANT NOTES

1. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduce size and amount of DNA.
2. All centrifugation steps should be carried out in a conventional table-top micro centrifuge at room temperature.
3. If precipitates formed in SL Buffer or SB Buffer, warm buffer to 37℃ until precipitates fully dissolve.

ADDITIONAL REQUIREMENTS

1. Pipettes and pipette tips
2. Micro-centrifuge (1.5 ml or 2 ml)
3. Water bath or heating block
4. Vortex mixer
5. Ethanol (96 – 100%)
6. Other general lab equipments

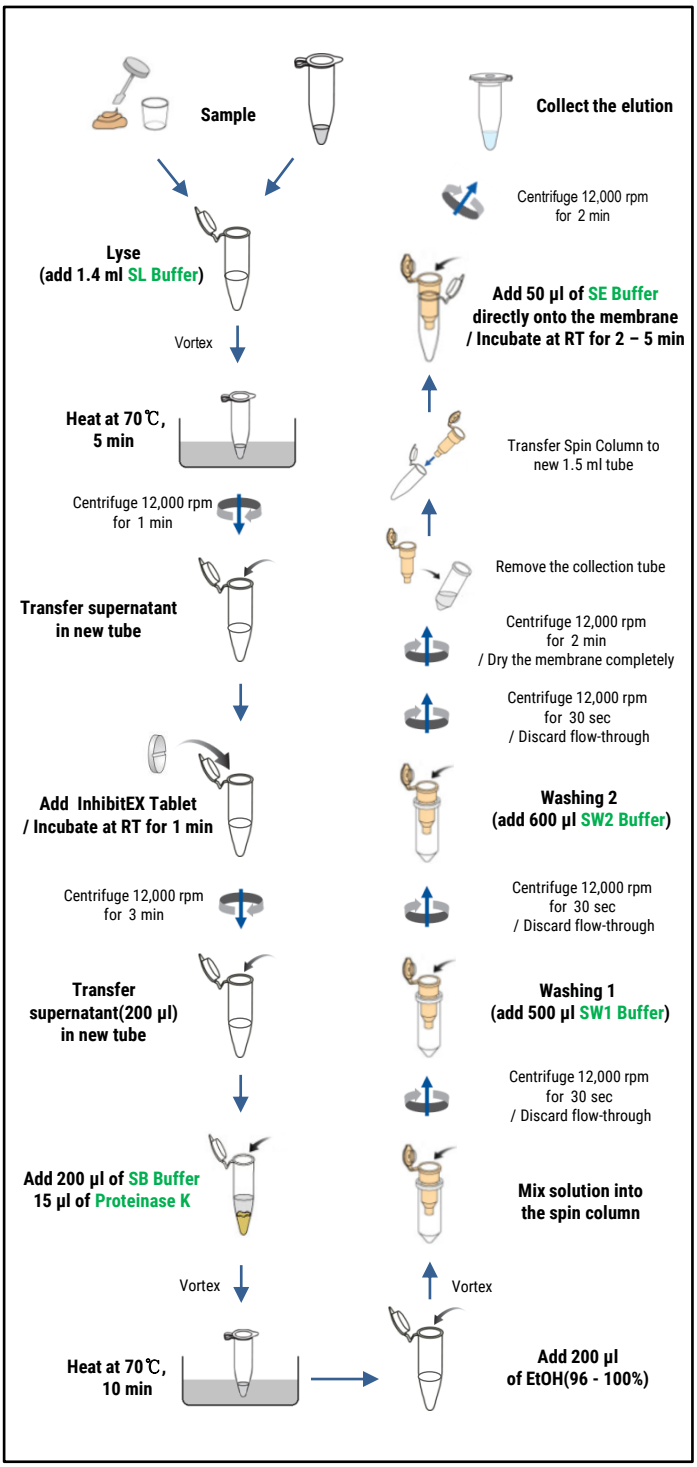
NOTICE BEFORE USE

The i-genomic Stool plus DNA Extraction Mini Kit is made of research use only. This product is not made of the diagnosis, prevention, or treatment of disease. All due care and attention should be exercised in the handling of the products. Do not use internally or externally in humans or animals. Please observe general laboratory precaution and utilize safety while using this kit.

APPLICATION

The i-genomic Stool plus DNA Extraction Mini Kit can purify large amounts of genomic DNA. PCR can be inhibited by excess total DNA. Depending on the individual stool sample, yield may be up to 10 μg total DNA (up to 200 ng/μl). In general, for optimum PCR results use the minimum amount of genomic DNA possible in PCR. The volume of elute used as template should not exceed 10% of the final volume of the PCR mixture.

DIAGRAM OF EXPERIMENT



PROTOCOL

1. Weigh 180-220 mg stool in a 2 ml micro-centrifuge tube (not provided) and place the tube on ice.

   NOTE : If the sample is liquid phase, pipet 200 µl into the microcentrifuge tube.

2. Add 1.4 ml SL Buffer, vortex continuously for 1 min until the stool sample is thoroughly homogenized.
3. Heat the suspension for 5 min at 70°C.

   NOTE : The lysis temperature can be increased to 95°C for cells that are difficult to lyse (such as Gram-positive bacteria).

4. Vortex for 15 s, centrifuge at 12,000rpm (~ 13,400 x g) for 1 min, transfer 1.2 ml supernatant into a new 2 ml centrifuge tube.
5. Add one InhibitEX Tablet to each sample and vortex until the tablet is completely suspended. Incubate the suspension for 1 min at room temperature to allow inhibitors to be adsorbed to the InhibitEX matrix.
6. Centrifuge at 12,000 rpm (~13,400 × g) for 3 min.
7. Transfer supernatant from the results of step 6 into a new 1.5 ml microcentrifuge tube. Repeat step 6 - 7.
8. Transfer 200 µl supernatant into a new 1.5 ml microcentrifuge tube. Add 15 µl proteinase K.
9. Add 200 µl SB Buffer, and vortex for 15 s.
10. Incubate at 70°C for 10 min to yield a homogeneous solution.

    NOTE : Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

11. Add 200 µl ethanol (96-100%), and mix thoroughly by vortex.

    NOTE : Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

12. Pipet the mixture into the Spin Column (in a 2 ml collection tube) and centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard flow-through and place the Spin Column into the collection tube.
13. Add 500 µl SW1 Buffer to Spin Column (Ensure that ethanol is added to SW1 Buffer before use), and centrifuge at 12,000 rpm (~13,400 × g) for 30 s, then discard the flow-through and place the spin column into the collection tube.
14. Add 600 µl SW2 Buffer to Spin Column (Ensure that ethanol is added to SW2 Buffer before use), and centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard the flow-through and place the spin column into the collection tube.
15. Repeat Step 14.
16. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min to dry the membrane completely.

    Note: The resident ethanol of SW2 Buffer may have some influence in downstream application.

17. Place the Spin Column in a new clean 1.5 ml microcentrifuge tube, and pipet 50 µl SE Buffer directly to the center of the membrane. Incubate at room temperature for 2-5 min, and then centrifuge for 2 min at 12,000 rpm (~13,400 × g). Collect the elution.

    Note: For enhancing recovery efficiency of gDNA, pipet the flow through from step 17 into spin column again, centrifuge for 2 min at 12,000 rpm (~13,400 × g). The pH value of eluted buffer will influence the eluting efficiency; we suggest to use SE Buffer or distilled water (pH 7.0 - 8.5) to elute gDNA. For long-term storage, eluting gDNA in SE Buffer and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

At iNtRON we pride ourselves on the quality and availability of our technical support. Our CRT center is organized by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of iNtRON products. If a iNtRON product does not meet your expectations, simply call your local distributor. If you have questions about product specifications or performance, please call iNtRON Technical Services or your local distributor.

TROUBLE SHOOTING GUIDE

This troubleshooting guide may be helpful in solving problems that may frequently arise. The scientists at iNtRON are always happy to answer any questions you may have about the information or protocol in this manual or other molecular biology applications.

Problem / Possible cause	Recommendation
Little or no DNA	
1) Sample stored incorrectly	• Sample should be stored 4℃ or -20 ℃.
2) Insufficient homogenization of stool sample	• Repeat the DNA extraction procedure with a new sample. Mixing well the sample and SL Buffer until the sample is thoroughly homogenized.
3) No alcohol added to the lysate before loading onto the Spin Column	• Repeat the extraction procedure with a new sample.
4) DNA not eluted efficiently	• To increase elution efficiency, pipet SE Buffer onto the spin column and incubate the column for 5 minutes at room temperature before centrifugation.
5) SW1 Buffer or SW2 Buffer prepared incorrectly	• Check that SW1 Buffer and SW2 Buffer concentrates were diluted with correct volumes of pure ethanol. Repeat the extraction procedure with a new sample.
A_{260}/A_{280} ratio for purified DNA is low	
1) Inefficient elimination of inhibitory substances due to insufficient mixing with InhibitEX matrix	• Repeat the DNA extraction procedure with a new sample. Be sure to mix the sample and InhibitEX Tablet until the sample is thoroughly homogenized.
2) Insufficient mixing with SL Buffer	• Repeat the procedure with a new sample. Be sure to mix the sample and SL Buffer immediately and thoroughly by pulse vortexing.
3) No alcohol added to the lysate before loading onto the spin column	• Repeat the purification procedure with a new sample.
4) SW1 Buffer or SW2 Buffer prepared with low-percentage ethanol	• Check that SW1 Buffer and SW2 Buffer concentrates were diluted with 96–100% ethanol. Repeat the purification procedure with a new sample.
A_{260}/A_{280} ratio for purified DNA is high	
1) High level of residual RNA	• Add 20µl of RNase A (20mg/ml) to the obtained DNA and incubate for 10 minutes at room temperature (15–25°C).
Little or no supernatant visible after precipitation of InhibitEX Tablet	
1) Insufficient centrifugal force	• With some samples, centrifugation to precipitate the InhibitEX Tablet may result in a pellet that is not sufficiently compact. In these cases, we recommend to increase the centrifugation time for precipitation of InhibitEX Tablet to 6 minutes.
DNA does not perform well in downstream applications	
1) BSA not added to PCR mixture	• When using the obtained DNA in PCR, for maximum PCR robustness add BSA to a final concentration of 0.1 µg/µl to the PCR mixture.
2) Too much DNA used in downstream reaction	• If the amount of total DNA is too high, PCR could be inhibited by excess total DNA. Reduce the amount of the obtained DNA used in the downstream reaction if possible.

ORDERING INFORMATION

Product Name	Amount	Cat. No.
Maxime™ PCR PreMix (i-Taq)	96 / 480tubes	25025 / 25026
Maxime™ PCR PreMix (i-StarTaq)	96 / 480tubes	25167 / 25165
Maxime PCR PreMix Kit (i-StarTaq™ GH)	96 / 480tubes	26050 / 26051
Sizer™-1000 plus DNA Marker	0.5 ml	24075
RedSafe™ Nucleic Acid Staining Solution	1 ml	21141
MEGAquick-spin™ Plus Total Fragment DNA Purification Kit	50 / 200 col.	17289 / 17290

Technical support : +82-505-550-5600

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