ISO 9001/14001 Certified Company

i-genomic Stool DNA Extraction Mini Kit

Cat. No. 17451 50 Columns

DESCRIPTION

The i-genomic Stool DNA Extraction Mini Kit provides fast and easy purification of stool DNA from fresh or frozen stool samples. i-genomic Stool DNA Extraction Mini Kit purified DNA is of high quality and is ideal for reliable use in PCR and other downstream enzymatic reactions. The i-genomic Stool DNA Extraction Mini Kit is designed to extract genomic DNA in 50 minutes from a small amount of stool matter from any of a variety of animals, including human, rat, bird, cat, and cow. The simple i-genomic spin procedure yields pure DNA ready for direct use in less than standardization and ease of use. Purification requires no phenol-chloroform extraction or alcohol precipitation, and involves minimal handling. DNA is eluted in low-salt buffer and is free of protein, nuclease, and other impurities or inhibitors. The purified DNA is ready for use in PCR and other enzymatic reactions, or can be stored at -20℃ for later use.

Genomic DNA obtained from stool samples provides a window into animal pathophysiology. For example, changes in the methylation patterns in fecal DNA may be a promising marker for human colorectal cancer screening. In rural watersheds, *E. coli* typing can help track the animal sources of fecal water pollution. In addition, PCR can be used to detect intestinal protozoan infections in avian wildlife populations, in humans, or for human biome studies.

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 DNA Extraction Mini Kit contains i-genomic Stool IR Spin Columns, a special tool provided in a convenient spin column form. i-genomic Stool IR Spin Columns efficiently absorb these substances early in the purification process so that they can easily be removed by a quick centrifugation step from stool samples.

KIT CONTENTS AND STORAGE

Label	Description	Contain
Buffer SPL	Pre-lysis Buffer	12 ml
Buffer SL	Lysis Buffer	12 ml
Buffer SB	Binding Buffer	12 ml
Buffer SWA (concentrate) [1]	Washing Buffer A	12 ml
Buffer SWB (concentrate) [2]	Washing Buffer B	10 ml
Buffer SE	Elution Buffer	20 ml
IR Spin Columns	Inhibitor Absorption Column	50 columns
Spin Columns (Violet O-ring color)	Inserted into the collection tubes (2.0 ml tubes)	50 columns
Collection Tubes (2.0ml tubes)	Additionally supplied.	50 tubes X 2ea
RNase A (Lyophilized powder) [3]	Dissolve with Pure DW 0.3 ml	3 mg
Proteinase K (Lyophilized powder)[4]	Dissolve with Pure DW 1.1 ml	22 mg

[1] Buffer SWA is supplied as concentrates. Add 28 ml of ethanol (96~100%) according to the bottle label before use.
[2] Buffer SWB is supplied as concentrates. Add 40 ml of ethanol (96~100%) according to the bottle label before use.
[3] After dissolving, store at -20℃. The RNase A is completely free of DNase activity.
[4] After dissolving, store at -20℃. After thawing, freshly use. We recommend to aliquot to small volume of Proteinase K.

QUALITY CONTROL

As iNtRON quality control program, the performance of iNtRON's products are monitored routinely on a lot-to-lot basis. The genomic DNA yield of i-genomic series Genomic DNA Mini Kit is tested by preparing various sample and assaying the genomic DNA yield spectrophotometrically. The quality of isolated genomic DNA is checked by restriction digestion, PCR, agarose gel electrophoresis, and spectrophotometry. i-genomic Stool DNA Mini Kit is tested to ensure the absence of DNase contamination. All buffers are each incubated with 1 mg pUC18 DNA for 6 hours at 37ºC. The DNA is then visualized by electrophoresis on an agarose gel and compared to a positive control to determine if any linear or nicked plasmid DNA is visible.

i-genomic series DNA Extraction Mini Kits

i-genomic series DNA Extraction Mini Kits provide nine kind of kits according to the type of samples as seen below table. These i-genomic series Kits provide a fast and easy way to purify DNA from various samples. The kits procedures provide pure genomic DNA for reliable PCR and Southern blotting in less than 1 ~ 2 hours. Purification requires no phenol or chloroform extraction or alcohol precipitation. Purified DNA extracted by i-genomic series Kits is eluted in low-salt buffer or water, ready for use in downstream applications, including PCR, RAPD analysis, AFLP analysis, RFLP analysis, Southern blotting, microsatellite analysis, SNP-genotyping, and quantitative, real-time PCR. Purified DNA has an A_{260}/A_{280} ratio of 1.7 ~ 2.0, indicating high purity of the DNA.

Nine Kinds of the i-genomic series DNA Extraction Mini Kits

Purpose		Product Name	Cat. No.	Samples
Specific Sample use (Big 5 Series)		**i-genomic CSI DNA Extraction Mini Kit**	17382	Micro-amount sample (Forensic Sample)
		**i-genomic Urine DNA Extraction Mini Kit**	17391	Urine / Urinal swab
		**i-genomic Food DNA Extraction Mini Kit**	17401	Vast kinds of Food
		**i-genomic Stool DNA Extraction Mini Kit**	17451	Stool / Stool swab
		**i-genomic Soil DNA Extraction Mini Kit**	17421	Soil / Soil bacteria
General Use (KIAGEN series)		**i-genomic CTB DNA Extraction Mini Kit**	17341	**C**ells **T**issues Gram(-) **B**acteria
		**i-genomic Blood DNA Extraction Mini Kit**	17351	Bloods
		**i-genomic BYF DNA Extraction Mini Kit**	17361	Gram(+) **B**acteria **Y**east **F**ungi
		**i-genomic Plant DNA Extraction Kit**	17371	Plant

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.

PRECAUTIONS AND SAFETY INFORMATION

All chemicals should be considered as potentially hazardous. When working with chemicals, always wear a suitable lab coat and disposable glove. Some buffer contain the chaotrophic salt which may be an irritant and carcinogen, so appropriate safety apparel such as gloves and eye protection should be worn. If a spill of the buffers occurs, clean with a suitable laboratory detergent and water. If the liquid spill contains potentially infectious agents, clean the affected area first with laboratory detergent and water, then with a suitable laboratory disinfectant. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products.

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.

RECOVERY OF PURIFIED DNA

Determination of concentration, yield, and purity DNA yield is determined from the concentration of DNA in the eluate, measured by absorbance at 260 nm. Absorbance readings at 260 nm should fall between 0.1 and 1.0 to be accurate. Sample dilution should be adjusted accordingly: for example, an eluate containing 25 ~ 50 ng DNA/ ml (A_{260} = 0.5 ~ 1.0) should not be diluted with more than 4 volumes of buffer. Use elution buffer or water (as appropriate) to dilute samples and to calibrate the spectro- photometer. Measure the absorbance at 260 and 280 nm, or scan absorbance from 220 ~ 320 nm (a scan will show if there are other factors affecting absorbance at 260 nm). Both DNA and RNA are measured with a spectrophotometer; to measure only DNA, a fluorimeter must be used. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. Pure DNA has an A_{260}/A_{280} ratio of 1.7 ~ 2.0. DNA purified by the i-genomic Stool Kit procedure is free of protein and other contaminants that can inhibit PCR or other enzymatic reactions. The purified DNA can be used immediately or safely stored in Buffer SE at –20°C for later use.

EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

i-genomic Stool DNA Mini Kit provides almost

- ✓ PBS (phosphate-buffered saline) may be required for some samples
- ✓ Ethanol (96 – 100%)
- ✓ Pipettes and pipette tips
- ✓ Micro-centrifuge (e. g., CENDORI™ S-12; iNtRON, Cat. No. 50100)
- ✓ Water bath or heating block, Vortex mixer
- ✓ Other general lab equipments

IMPORTANT POINTS BEFORE STARTING

▪ **Buffer SWA / SWB**

: Buffer SWA / SWB is supplied as concentrate. Before using for the first time, be sure to add 28 ml / 40 ml of absoulute ethanol (96 ~ 100%) to obtain a working solution.

▪ **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.

▪ **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.

▪ **Preheat a water bath or heating Block**

▪ **Centrifugation**

: All centrifugation steps are carried out at RT (15 ~ 25°C) in a micro-centrifuge.

COLUMN INFORMATION

▪ **i-genomic series Spin Column, applied to CAPS**

CAPS, Clean Automatic Packaging System

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

[1] Do not store the Column packs under completely dried conditions. It may be affected to DNA binding capacity. The Spin Columns are stable for over 1 year under these conditions

[2] Additional Collection Tubes (100 ea) are also supplied for your convenient handling.

PROTOCOL

: Refer to the "VISUAL PROTOCOL"

1. **Weigh 180 ~ 220 mg in a 1.5 ml tube and place the tube on ice.**
   Note : Exceeding the recommended optimal amount of starting material will result in inefficient lysis, resulting in low DNA yield and purity. To prevent thaw the frozen sample during transfer it, previously chill the spatula and 1.5 ml tube in liquid nitrogen. The freezing-thawing repetition of frozen sample will result in the DNA degradation.
2. **Add 200 ml of Buffer SPL to each stool sample. Vortex continuously for 1 min and incubate 70°C for 5 min.**
   Note : If the Buffer SPL become solid, incubate in 80°C for 10 min.
3. **Place the i-genomic Stool IR Spin Column into a new 1.5 ml tube (not supplied), and transfer supernatant promptly into the i-genomic Stool IR Spin column.**
4. **Centrifuge for 1 min at 13,000 rpm (RT), and remove the IR Spin Column from the 1.5 ml tube.**
5. **Add 200 ml of Buffer SL, 20 ml of Proteinase K and 5 ml of RNase A solution in 1.5 ml tube and mix by vortexing.**
6. **Incubate the lysates for 15 min at 65°C.**
   Note : For complete lysis, mix 5 or 6 times during incubation by inverting tube. The incubation time can be prolonged for more yields of DNA. The complete lysis let you see clear lysate.
7. **After lysis completely, add 200 ml of Buffer SB 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.**
8. **Add 250 ml of 80% ethanol to the lysate, and mix by pipetting or gently inverting 5 ~ 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.
9. **Pipette 750 ml of the mixture from step 8 into the spin column inserted in a 2.0 ml collection tube. Centrifuge at 13,000 rpm at RT for 1 min, and discard flow-through and collection tube altogether.**
   Note : The maximum volume of the spin column reservoirs is 800 ml. In case of the large volume of binding mixture, divide the binding mixture into halves and load the halve of binding mixture.
10. **Place the spin column into a new 2.0 ml collection tube (additionally supplied), add 700 ml of Buffer SWA to the spin column, and centrifuge at 13,000 rpm for 1 min. Discard the flow-through and reuse the collection tube in step 10.**
    Note : Ensure that 28 ml of ethanol (EtOH) has been added to Buffer SWA.
11. **Add 700 ml of Buffer SWB to the spin column, and centrifuge at 13,000 rpm for 1 min to dry membrane. Discard the flow-through and collection tube altogether.**
    Note : Ensure that 40 ml of absolute ethanol (EtOH) has been added to Buffer SWB.
    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.
12. **Place the spin column into a new 1.5 ml tube (not supplied), and 50 ml Buffer SE directly onto the membrane. Incubate for 1 min temperature, and then centrifuge at 13,000 rpm for 1 min to elute.**
    Note : Elution with 30 ml increases the final DNA concentration, but reduces overall DNA yield conventionally. Alternatively, if you need larger amounts of DNA, eluting with 100 ml increases generally overall DNA yield.
    Note : A new 1.5 ml tube can be used for the second elution step to prevent dilution of the first elute. Alternatively, the tube can be reused for the second elution step to combine the elutes.

TECHNICAL INFORMATION

EXPERIMENTAL INFORMATION

• *Genomic DNA Purification*

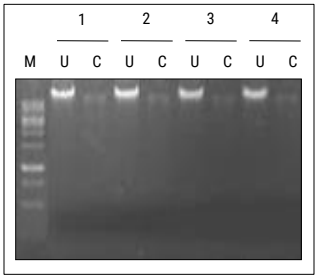


Fig. 1. Results of DNA purification
After eluting genomic DNA with 50 ml Buffer SE, each of 100 ng of DNA were used in DNA electrophoresis.
Panel 1, 2, i-genomic Stool DNA Extraction Mini Kit
Panel 3 , 4, Company A's product
Lane M, 1 kb Ladder DNA marker; **lane U,** Purified genomic DNA from Stool; **lane C,** Genomic DNA digested with *Eco*RI

Table 1. DNA purity by performing ratio absorbance measurements

	A_{260} (Average)	A_{280} (Average)	Purity (A_{260}/A_{280})	Yield (μg)
iNtRON	1.862	0.857	2.1	4.7
Company A	1.599	0.763	2.0	4.0

• *Genomic DNA PCR*

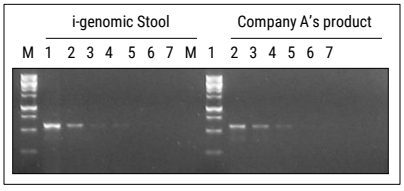


Fig. 2. GAPDH amplification
The housekeeping gene GAPDH (mammalian, 575 bp) was amplified with the purified DNA as template(5 ml) using *Maxime* PCR PreMix Kit (i-Taq) (Cat. No. 25025). The template DNA was serially diluted $10^0 \sim 10^{-6}$.
Lane M, 1 kb Ladder DNA Marker, **lane 1,** 10^0 of template DNA (10 ng); **lane 2,** 10^{-1} of template DNA; **lane 3,** 10^{-2} of template DNA; **lane 4,** 10^{-3} of template DNA; **lane 5,** 10^{-4} of template DNA; **lane 6,** 10^{-5} of template DNA; **lane 7,** 10^{-6} of template DNA

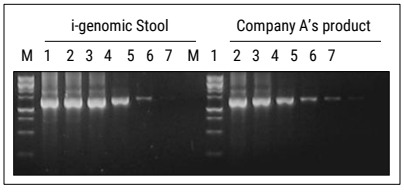


Fig. 3. 16S rRNA amplification
The 16s rRNA(bacteria, 1.6 kb) was amplified with the purified DNA as template(5 ml) using *Maxime* PCR PreMix Kit (i-Taq) (Cat. No. 25025). The template DNA was serially diluted $10^0 \sim 10^{-6}$.
Lane M, 1kb Ladder DNA Marker; **lane 1,** 10^0 of template DNA (10 ng); **lane 2,** 10^{-1} of template DNA; **lane 3,** 10^{-2} of template DNA; **lane 4,** 10^{-3} of template DNA; **lane 5,** 10^{-4} of template DNA; **lane 6,** 10^{-5} of template DNA; **lane 7,** 10^{-6} of template DNA

• Pathogen genomic DNA PCR

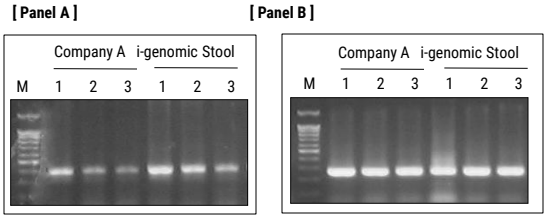


Figure 4. PCR results of *Clostridium perfringens* (CLOS) and *Canine parvovirus*(CPV)
CLOS(bacteria, 405 bp) and CPV(virus, 257 bp) were amplified with the purified DNA from infected stool sample as template (5 ml/each) using VeTeK™ CLOS Detection Kit (iNtRON, Cat. No.D10400) and VeTeK™ CPV Detection Kit (iNtRON, Cat. No.D10230).
Panel A , CLOS; **Panel B,** CPV
Lane M, 1 kb Ladder DNA Marker; **lane1 ~ lane 3,** Extracted genomic DNA of CLOS and CPV infected stool

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
Low DNA yield	Too much starting material	- Check the step 1 of protocol; Reduce the amount of starting material used.
	Reagents correctly were stored	- Check the "STOAGE"; Buffer SPL tend to become solid at RT. If Buffer SPL become solid, incubate in 80°C.
	Incomplete lysis	- Lysis time and buffer volume not correct for sample size.
	DNA inefficiently eluted	- Elute product with 100 ~ 200 $\mu\ell$ of the Buffer SE to obtain best result. - Depending on starting material size, decrease or increase volume of Buffer SE - Add Elution buffer to the center of the spin column to ensure that the Buffer SE completely covers the membrane.
	Buffer SWA, Buffer SWB did not contain 100% EtOH	- 100% EtOH must be added to Buffer SWA, Buffer SWB before use.
Problems in the downstream application	Ethanol carryover	- Ensure that during the Buffer SWB, the spin column is spun at maximum speed 1min to dry spin column membrane.

RELATED PRODUCTS

Product Name	Cat. No.
Maxime™ PCR PreMix (*i*-Taq)	25025
Maxime™ PCR PreMix (*i*-StarTaq)	25165
SiZer™-1000 DNA Marker	24074
RedSafe™ Nucleic Acid Staining Solution	21141

VISUAL PROTOCOL

Ⅰ Preparation step

1

Prepare the sample

Weigh 180 ~ 220 mg of sample

Transfer into 1.5 ml tube

Ⅱ Pre Lysis step

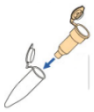
2

Add 200 ㎕ Buffer SPL

Mix by vortex vigorously

Incubate at 70℃ for 5 min

3



Place IR Spin Column into a new 1.5 ml tube

Transfer the prelysate into the IR Spin column.

4

Centrifuge 13,000 rpm for 1 min

Discard

Ⅲ Lysis step

5

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

Mix by vortexing vigorously (or mix by pipetting)

6

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

Ⅳ Binding step

7

Add 200 ㎕ of Buffer SB

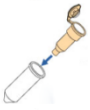
Mix by pipetting or inverting
Do NOT vortex

8

Add 250 ㎕ of 80% EtOH

Mix by pipetting or inverting
Do NOT vortex

9



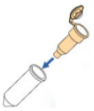
Place Spin Column into a new 2.0 ml Collection tube

Add 750 ㎕ of mixture (Lysate)

Discard

Ⅴ Washing step

10



Place Spin Column into a new 2.0 ml Collection Tube

Add 700 ㎕ of Buffer SWA
Note :
Ensure that 28 ml of EtOH has been added to buffer SWA

Centrifuge 13,000 rpm for 1 min

Discard the flow-through

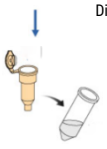
11

Add 700 ㎕ of Buffer SWB
Note :
Ensure that 40 ml of EtOH has been added to buffer SWB

Centrifuge 13,000 rpm for 1 min

Discard the flow-through

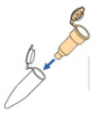
Centrifuge 13,000 rpm for 1 min



Discard the flow-through

Ⅵ Elution step

12



Transfer Spin Column to new 1.5 ml tube

Add 50 ㎕ of Buffer SE directly onto the membrane

Incubate at RT for 1 min

Centrifuge 13,000 rpm for 1 min

Recover the final eluate (gDNA)

Discard