

TECHNICAL HANDBOOK

XNAPS Stool DNA Flexspin Kit

Catalog Number P1023A
P1023B

Total DNA purification from
Stool samples

Version 2008

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All technical literature and related information are available on the website: www.renogenbio.com

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Kit Contents

Catalog Number	P1023A	P1023B
Kit Size (preps)	50	100
Lysis buffer LB	30 ml	60 ml
Denaturation buffer DB	20 ml	40 ml
Wash buffer WB	24 ml	24 ml x 2
Nuclease-free water	20 ml	20 ml
DNA binding resin	5 ml	10 ml
RNAse A solution	0.25 ml	0.5 ml
Filter columns	50	100
Collection Tubes	50	100
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Storage Conditions

Upon received, store the RNase A solution at -20°C. Store the DNA Binding Resin at 2-8°C. All other buffers and components can be stored at room temperature for one year without any reduction of performance.

Quality Control

In addition to routine monitoring and detection of the kit components, the performance of XNAPS stool DNA flexspin kits are tested on a lot-to-lot basis by purification of total nucleic acid from 50mg of frozen stool sample. The yield and purity of purified DNA is checked by agarose gel electrophoresis, spectrophotometrical analysis.

Safety Precautions

Although no toxic reagents are contained in the serial XNAPS kits, all chemicals should be considered as potentially hazardous. All due care and attention should be exercised in handling the materials and reagents in the kit. We recommend users always wear laboratory coat, safety glasses, and gloves. In the case of contact with skin or eyes, wash immediately with a large amount of water.

Technical Assistance

We encourage our customers to contact us by any means of telephone, fax, mail/email. Our experienced staff are always ready to assist you about any questions and problems derived from our products. Also, you can find most of the information and data of Renogen products from our website.

Contacting information:

Web: www.renogenbio.com

Tel: 1-866-712-4412(Toll free)

Email: services@renogenbio.com

Introduction

XNAPS stool DNA minispin kit provides a fast, simple, and efficient method for isolating total nucleic acid or DNA from fresh or frozen stool samples. The nucleic acid may derive from any sources, such as cellular, bacterial, viral, in the stool sample. The kit combines a two-step chemical/enzyme lysis procedure with an innovative chromatographic absorbent resin which, while removing efficiently the impurities and PCR inhibitors from stool samples, preferentially binds nucleic acid with high capacity. After removal of residual chemicals and impurities by strong washing, pure DNA is eluted in water or TE buffer.

The purified genomic DNA is immediately ready to use in various downstream applications, such as PCR, restriction enzyme digestion, sequencing.

Key Features

1. All the cellular DNA, microorganismal DNA and microparasitic DNA in the sample can be purified simultaneously.
2. Flexibility: The process can be scaled up or down for different amounts of starting samples.
3. Rapidity: All the processing steps can be completed within 30 minutes.
4. High yield and purity: Up to 50 µg of DNA with OD₂₆₀/OD₂₈₀ between 1.7-1.9 can be extracted from 100 mg of stool sample.
5. Safety for handling, shipping and storage: No phenol extraction, no ethanol precipitation, no toxic chaotropic salts.

Quick Protocol (For experienced users)

The principle of XNAPS stool DNA flexspin kit is totally different from the methods of other suppliers. Do not exchange or replace the components of XNAPS stool DNA flexspin kit with components from any other suppliers.

1. Transfer 100 mg of stool sample to a 1.5 ml tube.
2. Add 600 µL of lysis buffer LB. Homogenize thoroughly by vortexing. Incubate at 60°C for 10 minutes.
3. Spin for 5 minutes. Transfer 400 µl of supernatant into a new 1.5 ml tube.
4. Add 5 µl of RNase A solution. Incubate at 37 °C for 15 minutes
5. Add 10 µl of Proteinase K solution. Incubate at 60°C for 10 minutes.
6. Add 350 µl of denaturing buffer DB. Mix thoroughly. Place the tube on ice for 5 minutes. Spin for 10 minutes. Pour the supernatant to a filter column.
7. Add 100 µl of DNA binding resin. mix the resin thoroughly by pipetting up and down. Stand the tube at room temperature for 1 minute.

8. Spin for 1 minute. Discard the flowthrough.
9. Add 800 µl of WB. Spin for 1 minute. Discard the flowthrough.
10. Repeat step 9 twice.
11. Discard the flowthrough. Spin for 1 minute.
12. Transfer the filter column to a new 1.5ml tube.
13. Add 100-200 µl of nuclease-free water into the filter column. Stand at room temperature for 1 minute. Spin for 1 minute.
14. Store the DNA at -20°C.

Detailed Protocols

The principle of XNAPS stool DNA flexspin kit is totally different from the methods of other suppliers. Do not exchange or replace the components of XNAPS stool DNA flexspin kit with components from any other suppliers.

Materials to be supplied by the user

Microcentrifuge capable of 14000 x g
60°C water bath or heating block
Proteinase K solution (20mg/ml)
Ethanol(>95%)
Sterile 1.5ml microcentrifuge tubes

Prior to starting:

1. Add 96 ml of ethanol(>95%) to wash buffer WB.
2. Preheat a water bath or heating block to 60°C.
3. Examine the buffers in the kit. Buffers may form precipitation in low storage temperature. If there is precipitate, warm at up to 60°C until the solution becomes clear.

Tab.1 Needed solutions for different amounts of stool samples

Weight of sample	50 mg	100 mg	150 mg	200 mg	250 mg	>250 mg
LB(μl)	600	600	600	600	600	Flexible*
DB(μl)	350	350	350	350	350	Flexible*
WB(ml)	2.4	2.4	2.4	2.4	2.4	Flexible*
Resin(μl)	50	100	125	150	200	Flexible*
Elution volume(μl)	100	100-200	100-200	100-200	100-200	200

*:Proportional to the volume for 250 mg of sample

The following protocol is for 100 mg of stool sample. For different amounts of samples, adjust the needed solutions according to Tab. 1.

I. Preparation of stool sample

Weigh and Transfer 100 mg of stool sample to a 1.5 ml centrifuge tube.

II. Preparation of Lysate

1. Add 600μl of lysis buffer LB to the tube. Close the tube tightly and homogenize thoroughly by vortexing for 30 s. *If LB is precipitated or cloudy*

before use, heat the solution up to 60°C until it becomes clear.

2. Incubate the tube at 60°C for 10 minutes.
3. Vortex for 5 seconds. Centrifuge at maximum speed for 5 minutes.
4. Transfer 400 μl of the supernatant into a new 1.5 ml tube.
5. Add 5 μl of RNase A solution. Incubate at 37 °C for 15 minutes.
6. Add 10 μl of Proteinase K solution. Incubate at 60°C for 10 minutes.
7. Add 350μl of Denaturing buffer DB. Close the tube and mix thoroughly by inverting the tube 4-5 times. Place the tube on ice for 5 minutes.
8. Centrifuge the lysate at maximum speed in a microcentrifuge for 10 minutes.

The solution should become cloudy with white precipitates.

Clear supernatant should be formed.

III. Isolation of DNA

1. Insert a filter column into a 2 ml collection tube.
1 set for each sample.
2. Decant gently the supernatant into the filter column.
3. Add 100 µl of DNA binding resin into the column.
Mix the resin completely by pipetting up and down. Incubate at room temperature for 1 minute.

Notice: *Shake or vortex the bottle of DNA binding resin to resuspend the resin completely before transferring.*

4. Centrifuge at maximum speed for 1 minute.
Discard the flowthrough from the collection tube.
5. Add 0.8 ml of wash buffer WB into the tube.
Centrifuge at maximum speed for 1 minute.
Discard the flowthrough.
6. Repeat the wash step twice.
7. Discard the flowthrough. Re-insert the column into the collection tube. Centrifuge at maximum speed for 1 minute.
8. Transfer the column into a new 1.5 ml tube.

The resin may pellet against the side of the filter column because of the fixed angle of rotors for most tabletop centrifuges.

9. Add 100-200 µl of nuclease-free water into the column. The water should be applied to immerse the highest portion of pellet resin. Stand the tube at room temperature for 1 minute. Centrifuge at maximum speed for 2 minutes.

Tips: *Use prewarmed(60°C) water can increase the yield by 20-30%.*

10. The purified total DNA is ready for downstream applications. Or, store the purified DNA at -20° for later use.

Yield and purity Examination

Both spectrophotometrical analysis and agarose gel electrophoresis are recommended for the yield and purity determination of the purified DNA. To determine the concentration of DNA by spectrophotometer, the following formula should be used :

$$[\text{DNA}] (\mu\text{g/ml}) = A_{260} \times 50 \times D,$$

where D is the dilution factor.

The yield of DNA can be calculated by multiplying the concentration by the volume of DNA solution.

The DNA purified by XNAPS stool DNA flexspin kit should be of high purity with the ratio of OD_{260}/OD_{280} between 1.7-1.8.

Supplementary Information

Enhancement of PCR performance

For some stool samples, contaminants may be co-extracted with DNA. These contaminants may interfere with downstream PCR. To enhance the PCR performance, try the following hints:

1. Add bovine serum albumin (BSA) to PCR mixtures to a final concentration of 0.1-1.0 $\mu\text{g}/\mu\text{l}$. BSA allows positive amplifications in PCR. Using DNA purified from highly contaminated starting materials as template, BSA can enhance the downstream PCR performance effectively.
2. Dilute the extracted DNA 5-10 fold before amplification.
3. Re-cleanup the extracted DNA.
4. Use less stool samples(50 mg) for extraction.

Troubleshooting

Problem	Possible Causes	Comments
Low yield or no DNA in elute	Poor lysis	Lyse sample at 75° for 30 mins.
	Ethanol omitted from Wash Buffer	Add ethanol as described
	Poor elution	Use prewarmed water (>60°C) to resuspend the resin and incubate for 2 minutes
OD ₂₆₀ /OD ₂₈₀ is too low	Protein contamination	Increase the lysis time with proteinase K
		Do another 3 washes with 70% of ethanol as in step 4 of Isolation of DNA
OD ₂₆₀ /OD ₂₃₀ is too low	Salts contamination	Do another 3 washes with 70% of ethanol as in step 4 of Isolation of DNA

Product Use Limitations

XNAPS stool DNA flexspin kit is developed and sold for research purpose only. It is not to be used for human diagnostic or drug purposes or to be administered to humans and animals. The user is responsible to validate the performance of the system for any specific applications.

Product warranty

Renogen guarantees the performance of all products for applications as described in the technical handbook. If any product fails to perform as described due to any reason, other than misuse, we will replace it free of charge or refund the purchase price.

We reserve the rights to change, alter, or modify our products to enhance its performance and design. If you have any concerns about Renogen products and services, please contact us by telephone, fax, mail, or email.

Ordering Information

Customers in USA and Canada

To place an order, please use any of the following ways:

Phone: 1-866-712-4412(Toll free) Mon.-Fri 8:00am-5:00pm (EST)
Fax: 1-651-204-9348
Mail: #310, 2386 East Mall Vancouver, BC, V6T 1Z3 Canada

Customers out of USA and Canada

Please contact our authorized international distributors and local representatives. In areas without our distributors and representatives, following options are available:

Phone: 1-651-204-0326 Mon.-Fri 8:00am-5:00pm (EST)
FAX: 1-651-204-9348
Mail: #310, 2386 East Mall Vancouver, BC, V6T 1Z3 Canada

Online Ordering

Ordering for all of our products from any places is available, 24 hours/day, 7 days/week. Online ordering is fast, and convenient. Please log on www.renogenbio.com for detailed information.