

XNAPS soil DNA flexspin kit

Catalog Number P1035A
P1035B

Total DNA purification from
Soil samples

Renogen Biolab Inc.
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Kit Contents

Catalog Number	P1035A	P1035B
Kit Size	50 preps	100 preps
Lysis buffer LB	30 ml	60 ml
Denaturation buffer DB	20 ml	40 ml
Wash buffer WB	60 ml	60 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, RNase A solution should be stored 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 soil DNA flexspin kits

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are tested on a lot-to-lot basis by purification of total nucleic acid from 150mg of soil 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.

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Introduction

XNAPS soil DNA flexspin kit offers a fast, simple, and efficient method for isolating total nucleic acid or total DNA from flexible amounts of soil samples, such as compost, sediment, and manure. The modified 2-step enzymatic lysis procedure can lyse efficiently all types of microorganism in the soil samples, including bacteria, fungi, algae, actinomycetes, and microparasite. The released nucleic acid binds specifically with the resin in the aqueous environment. Impurities are washed away by batch and column washing, high quality nucleic acid is eluted by pure water. This novel procedure overcomes many disadvantages, such as time-consuming methods, toxic components, low yield, as compared to other commercial kits.

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

Key Features

1. **Flexibility:** The processing can be scaled up or down according to starting samples

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2. **Efficiency:** Very little amount of DNA can be extracted successfully.
3. **High yield and purity:** 10 µg of DNA with OD₂₆₀/OD₂₈₀ between 1.7-1.8 can be extracted from 150 mg of soil sample.
4. **Safety** for handling, shipping and storage: No phenol extraction, no ethanol precipitation, no toxic chaotropic salts.

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Quick Protocol (For experienced users)

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

1. Transfer up to 150 mg of soil 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 3 minutes. Transfer 350 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.
7. Place the tube on ice for 5 minutes. Spin for 10 minutes.
8. Pour the supernatant to a new 1.5 ml tube. Add 100 µl of DNA binding resin. Invert the tube 4-5 times to resuspend the resin thoroughly. Stand the tube at room temperature for 1 minute.
9. Spin for 10 seconds. Discard the supernatant.
10. Add 1 ml of WB to resuspend the resin. Spin for 10 seconds. Discard the supernatant.
11. Repeat the step 10 once.
12. Resuspend the resin in 400 µl of WB. Transfer the mixture into the filter column. Spin for 1 minute. Discard the flowthrough.
13. Spin for 2 minutes.
14. Transfer the filter column to a new 1.5ml tube.
15. Add 100 µl of nuclease-free water into the filter column. Stand at room temperature for 1 minute.
16. Spin for 2 minutes.

Detailed Protocols

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

Ethanol

Proteinase K solution (20mg/ml)

Sterile 1.5ml microcentrifuge tubes

Prior to starting:

1. Add 60 ml of ethanol to each bottle of 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 a precipitate, warm at up to 60°C until the solution becomes clear.

Tab.1 Needed solutions for different amounts of soil 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	50	100	100	150	Flexible*
Elution volume(μl)	50	50	100	100	100-200	200

*:Proportional to the volume for 250 mg of sample

The following protocol is for 150 mg of soil sample. For different amounts of samples, adjust the needed solutions according to Tab. 1.

I. Preparation of soil sample

Transfer up to 150 mg of soil 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 3 minutes.
4. Transfer 350 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.
The solution should become cloudy with white precipitates.
8. Centrifuge the lysate at maximum speed in a microcentrifuge for 10 minutes.

Clear supernatant should be formed.

III. Isolation of DNA

1. Decant gently the supernatant into a new 1.5 ml tube.
2. Add 100 µl of DNA binding resin into the tube. Invert the tube 4-5 times to resuspend the resin completely. Incubate at room temperature for 1 minute.

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

3. Centrifuge for 10 seconds. Discard the supernatant from the tube.
4. Add 1 ml of wash buffer WB into the tube. Resuspend the resin thoroughly by pipetting up and down or by vortexing briefly. Centrifuge for 10 seconds. Discard the supernatant.
5. Repeat the wash step for once more. Remove the supernatant completely.
6. Insert a filter column into a collection tube.
7. Resuspend the resin in 400 µl of WB. Transfer the mixture into the filter column. Centrifuge at maximum speed for 1 minute

8. Discard the flowthrough. Re-insert the column into the collection tube. Centrifuge at maximum speed for 2 minutes.
9. 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.
10. Add 100 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%.*
11. Store the purified DNA at -20°C or below.

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 soil 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 soil samples, contaminants, such as humic acid, 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 µg/µl. 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 soil samples(50 mg) for extraction.

Troubleshooting

Problem	Possible Causes	Comments
Low yield or no DNA in elute	Poor lysis	Lyse sample at 65° for 30 minutes.
	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
Eluted DNA solution is brown	Too high content of humic acid in sample, or insufficient washing.	Do another 3 washes with 70% of ethanol as in step 4 of Isolation of DNA
		Re-cleanup of extracted DNA
		Pre-removal of humic acid before isolation of DNA
	Protein contamination	Increase the lysis time with proteinase K

OD ₂₆₀ /OD ₂₈₀ is too low		Do another 3 washes with 70% of ethanol as in step 4 of Isolation of DNA
OD ₂₆₀ /OD ₂₃₀ is too low	Salts or humic acid contamination	Do another 3 washes with 70% of ethanol as in step 4 of Isolation of DNA

Product Use Limitations

XNAPS soil 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
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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.