

TECHNICAL HANDBOOK**XNAPS Body Fluid DNA Flexspin Kit**

**Catalog Number P1026A
P1026B**

**For purification of total DNA from
flexible amounts of
plasma,
serum
body fluids**

Version 2009

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

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Kit Contents

Kit Catalog Number	P1026A	P1026B
Kit Size(preps)	50*	100*
Lysis Buffer LB	15 ml	30 ml
Denaturation Buffer DB	20 ml	40 ml
Column Wash Buffer WB	12 ml	24 ml
DNA Binding Resin	5 ml	10 ml
Nuclease-free Water	20 ml	20 ml
Filter Columns	50	100
Collection Tubes	50	100
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*: NP1026A/B contains enough reagents for 50/100 preps from 0.2 ml of body fluid.

Storage Conditions

Store the DNA Binding Resin at 4-8°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 monitor and detection for the kit components, the performance of XNAPS body fluid DNA flexspin kits are tested on a lot-to-lot basis by purification of total DNA from 200 µl of saliva. The yield and purity of purified genomic DNA is checked by agarose gel electrophoresis, spectrophotometrical analysis.

Safety Precautions

Although no toxic reagents are contained in XNAPS body fluid DNA flexspin kit, 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:

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Introduction

XNAPS body fluid DNA flexspin kit provides a fast, simple, and efficient method for isolating total DNA from flexible volumes of plasma, serum, body fluids (urine, saliva, sputum, cerebrospinal fluid), or other aqueous solutions. The system combines a modified enzyme lysis procedure with an innovative chromatographic absorbent resin which preferentially binds DNA with high capacity. The novel procedure provided by the kit overcomes many disadvantages, such as time-consuming, toxic components, low yield, as compared to other commercial kits. The purified DNA is immediately ready to use in various downstream applications, such as PCR, restriction enzyme digestion, sequencing.

Features:

1. **Flexibility:** Processing can be adjusted according to the volume of starting samples.
2. **Rapidity:** All processing steps can be completed in 10min after sample lysis.
3. **High yield and purity:** OD₂₆₀/OD₂₈₀ of purified DNA is between 1.8-2.0.
4. **Safety for handling, shipping and storage:** No phenol extraction, no ethanol precipitation, no toxic chaotropic salts.
5. Complete removal of contaminants and inhibitors.

Quick Protocol (For experienced users)

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

1. Transfer 200 µl of body fluid to a 1.5 ml tube.
2. Add 200 µl of buffer LB and 10 µl of Proteinase K solution. Vortex for 5 seconds. Incubate at 55°C for 10-30 minutes.
3. Add 350µl of buffer DB. Mix by vortexing briefly. Incubate on ice for 5 minutes.
4. Centrifuge at maximum speed for 5 minutes.
5. Transfer the supernatant to a filter column.
6. Add 100 µl of DNA binding resin. Mix thoroughly. Stand at room temperature for 1 minute.
7. Centrifuge at maximum speed for 1 minute. Discard the flowthrough.
8. Add 600µl of buffer WB. Centrifuge at maximum speed for 1 minute. Discard the flowthrough.
9. Repeat the washing once.
10. Centrifuge at maximum speed for 1 minute.
11. Transfer the column to a new 1.5ml tube. Add 100-200 µl of nuclease-free water. Stand at room temperature for 1 minute. Centrifuge for 2 minutes.
12. Store the DNA at -20°C.

Detailed Protocols

The principle of XNAPS body fluid DNA purification system is totally different from the methods of other suppliers. Do not exchange or replace the components with components from any other suppliers.

Starting material

XNAPS body fluid DNA flexspin kit can be used with flexible amounts of plasma, serum, or body fluids(urine, saliva, sputum, cerebrospinal fluid).All samples may be either fresh or frozen. Frozen samples should be thawed quickly in a 37°C water bath with mild agitation before beginning the procedure.

Materials to be supplied by the user

Microcentrifuge capable of 14000 x g
Centrifuge capable of 10000 x g
55°C water bath or heating block
Ethanol(>95%)
Sterile 1.5ml microcentrifuge tubes
Sterile centrifuge tubes
Vortex mixer
Proteinase K solution (20mg/ml)
(Optional)RNAse A solution(10mg/ml)

Prior to starting:

1. Add 48 ml/96 ml of ethanol(>95%) to wash buffer WB of P1026A/P1026B.
2. Preheat a water bath or heating block to 55°C
3. Ensure that all solutions are at room temperature prior to use. If precipitates have formed, warm the solutions at 55°C until they become clear.

I. Isolation of total DNA from <0.2ml of starting material

1. Transfer up to 200 µl of starting material into a sterile 1.5ml tube.
2. Add 200µl of Lysis Solution LB and 10 µl of Proteinase K solution (supplied by user) to the tube. Mix thoroughly by vortexing for 5 seconds. Incubate the tube at 55°C for 10-30 minutes.
3. (Optional) Add 5 µl of RNAse A solution (supplied by user) into the tube. Incubate at room temperature for 15 minutes.
4. Add 350µl of denaturation solution DB. Close the tube and mix thoroughly by vortexing. Incubate the tube on ice for 5 minutes. *The solution should become cloudy. The fluffy white material contains cell debris, proteins and SDS.*
5. Centrifuge the lysate at maximum speed in a microcentrifuge for 5 minutes. *Clear supernatant should be formed, although a few white precipitants may float on the top.*
6. Insert one filter column into one 2ml collection tube for each sample.
7. Decant gently the clear supernatant from step 5 into the filter column.
8. Add 100 µl of DNA binding resin into the filter column. Mix thoroughly by pipetting up and down. Stand at room temperature for 1 minute.

Note: Resuspend the resin thoroughly by vortexing or shaking before transferring.

9. Centrifuge at maximum speed in a microcentrifuge for 1 minute. Discard the flowthrough from the collection tube. Reinsert the column into collection tube.
10. Add 600 µl of wash buffer WB. Centrifuge at maximum speed in a microcentrifuge for 1 minute. Discard the flowthrough.
11. Repeat the washing (step 10) once. Discard the flowthrough from the collection tube.
12. Reinsert the column into the collection tube. Centrifuge at maximum speed for 1 minute.
13. Transfer the column to a new, sterile 1.5ml microcentrifuge tube. Discard the collection tube.
14. Add 100-200 µl of nuclease-free water into the center of the column. Stand at room temperature for 1 minute. Centrifuge at maximum speed for 2 minutes. *While 200 µl elution makes higher yield, 100 µl elution will have higher concentration of DNA.*
15. The purified DNA is ready for downstream applications. Or, store the purified DNA at -20°C for later use.

II. Isolation of total DNA from >0.2ml of starting material

1. Transfer starting material, e.g. 500 µl of urine, into a centrifuge tube. *The volume of the centrifuge tube should be at least 4 times larger than the volume of starting material.*
2. Add equal volume of Lysis Solution LB, e.g. 500 µl of LB, and 1/20 volume of Proteinase K solution (supplied by user), e.g. 25 µl of Proteinase K, to the tube. Vortexing for 5 seconds to mix the solution thoroughly. Incubate the tube at 55°C for 10-30 minutes.
3. (Optional) Add 10 µl of RNase A solution (supplied by user) into the tube. Incubate at room temperature for 15 minutes.
4. Add equal volume of denaturation solution DB, e.g. 1000 µL of DB. Close the tube and mix thoroughly by vortexing. Incubate the tube on ice for 5 minutes. *The solution should become cloudy. The fluffy white material contains cell debris, proteins and SDS.*
5. Centrifuge the lysate at 10000 x g for 10 minutes. *Clear supernatant should be formed, although a few white precipitants may float on the top.*
6. Transfer the supernatant to a new centrifuge tube. Add 100 µl of DNA binding resin. Close the tube and mix thoroughly by inverting the tube 4-5 times.

Incubate the tube at room temperature with gentle agitation for 5-10 minutes on a rotator.

Note: Resuspend the resin thoroughly by vortexing or shaking before transferring.

7. Centrifuge at 6000 x g for 1 minute. Decant the supernatant from the tube. While in centrifugation, insert one filter column into one 2ml collection tube.
8. Resuspend the pellet with 600 µl of wash buffer WB by vortexing or pepping.
9. Transfer the suspension into the filter column. Centrifuge at maximum speed in a microcentrifuge for 1 minute. Discard the flowthrough from the collection tube. Reinsert the column into collection tube.
10. Add 600 µl of column wash buffer WB. Centrifuge at maximum speed in a microcentrifuge for 1 minute. Discard the flowthrough.
11. Repeat the washing (step 10) once. Discard the flowthrough from the collection tube.
12. Reinsert the column into the collection tube. Centrifuge at maximum speed for 1 minute.
13. Transfer the column to a new, sterile 1.5ml microcentrifuge tube. Discard the collection tube.
14. Add 100-200 µl of nuclease-free water into the center of the column. Stand at room temperature for 1 minute. Centrifuge at maximum speed for 2

minutes. While 200 µl elution makes higher yield, 100 µl elution will have higher concentration of DNA.

15. The purified DNA is ready for downstream applications. Or, store the purified DNA at -20°C 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 body fluid DNA flexspin kit should be of high purity with the ratio of OD_{260}/OD_{280} between 1.8-2.0.

Troubleshooting

Problem	Possible Causes	Comments
Low yield or no DNA in elute	Too large volume of starting material	Use less amount of volume
	Ethanol omitted from Wash Buffer	Add ethanol as described
	Poor elution	Add prewarmed water (>60°C) and incubate for 3 minutes
	sample is too old	Use fresh sample
RNA contamination	Insufficient RNase A digestion	increase the incubation time or add 1 µl of RNase A to the purified DNA
Spin column is clogged	Too large sample volume	Centrifuge for a longer period of time until the lysate or solution passes through the column
	The centrifuge force is not high enough.	Centrifuge for a longer period of time until the lysate or solution passes through the column.

Product Use Limitations

XNAPS body fluid 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 Biolab 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.