

TECHNICAL HANDBOOK

XNAPS Cell/tissue gDNA Flexspin Kit

Catalog Number P1017A
P1017B

For purification of genomic DNA from
cultured cell
animal tissue
mouse tail

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	P1017A	P1017B
Kit Size (preps)	50	100
Lysis Buffer LB	20 ml	40 ml
Denaturation Buffer DB	20 ml	40 ml
Wash Buffer WB	12 ml	24ml
RNAse A Solution	0.25 ml	0.5 ml
Nuclease-free Water	20 ml	20 ml
DNA Binding resin	5ml	10 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. 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 cell/tissue DNA flexspin kits are tested on a lot-to-lot basis by purification of genomic DNA from 30mg of frozen animal

The yield and purity of purified genomic DNA is checked by agarose gel electrophoresis, spectrophotometrical analysis and restriction endonuclease digestion.

Safety Precautions

Although no toxic reagents are contained in XNAPS cell/tissue gDNA 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 cell/tissue DNA flexspin kit is designed to extract and purify genomic DNA from flexible amounts of animal cells or tissues. The amount of starting samples can range from 10^3 to 10^7 of cells, or from <1mg to 100mg of tissue, or even more. The system combines a modified enzyme lysis procedure with an innovative chromatographic absorbent resin which preferentially binds DNA with high capacity. The cells or tissues are digested completely by proteinase K in lysis buffer, which eliminates the need of grind or homogenization. Contaminating proteins and impurities are further denatured by buffer DB and removed by centrifugation. The DNA binds with resins in aqueous condition. After chemicals are removed by washing, pure DNA is eluted in water or buffer TE.

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

Features:

1. **Flexibility:** The starting tissues can range from <1mg to 100mg, even more.
2. **Speed:** All the processing steps can be completed within 20 minutes.
3. **High yield and purity:** Up to 10 μ g of genomic DNA with a ratio of $OD_{260}/OD_{280} > 1.8$ can be extracted from 10mg of animal tissues.
4. **Safety for handling, shipping and storage:** No phenol extraction, no ethanol precipitation, no toxic chaotropic salts.
5. The molecular weight of purified genomic DNA is 10-20 kb, suitable for downstream manipulations

Protocols

The principle of XNAPS cell/tissue gDNA purification system is totally different from the methods of other suppliers. Do not exchange or replace the components of XNAPS cell/tissue gDNA flexspin kit with components from any other suppliers.

Starting material

XNAPS cell/tissue gDNA Flexspin kit can be used with from 10^3 to 10^7 of cells, from <1mg to 100mg of tissue, or 1 cm of mouse tail. 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
55°C water bath or heating block
Ethanol(>95%)
Proteinase K solution (20mg/ml)
Sterile 1.5ml microcentrifuge tubes
Vortex mixer
Sterile pipette tips
Ice

Prior to starting:

1. Add 96 ml of ethanol(>95%) to wash buffer WB.
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 the solutions become clear.

I. DNA isolation from cultured cells

(This protocol can be applied for DNA isolation from up to 5×10^6 of cultured cells. For more cells, solutions and buffers should be adjusted proportionally. See the supplementary information in this manual for the preparation of cultured cells).

1. **Add 400 μ l of lysis buffer LB to the tube containing the cell pellet. Resuspend the cells by vortexing or pipetting up and down. The solution should become clear.**
2. (Optional) **Add 10 μ l of Proteinase K to the tube.** Mix well by vortexing. **Incubate the tube at 55°C for 15-30 minutes.**
3. **Add 5 μ l of RNase A solution.** Mix well by vortexing. **Incubate at 37°C for 15 minutes.**
4. **Add 350 μ l of Denaturing solution DB. Close the tube and mix thoroughly by vortexing for 10 seconds.** *The solution should become cloudy and less viscous. The fluffy white material contains cell debris, proteins and SDS.*
5. **Incubate the tube on ice for 5 minutes. Centrifuge the cell lysate at maximum speed in a microcentrifuge for 5 minutes.** *Clear supernatant should be formed. If not, incubate the lysate on ice for another 10 minutes and repeat the centrifugation step.*
6. Insert a spin column into a collection tube. **Transfer the clear supernatant from above step into the spin column.**
7. **Add 100 μ l of DNA binding resin. Mix the suspension thoroughly by pipetting up and down for 4-5 times. Stand the tube at room temperature for 1 minute.**

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

8. **Centrifuge at maximum speed for 1 minute.** Discard the flowthrough from the Collection tube. Reinsert the Spin Column into the Collection Tube.
9. **Add 600 μ l of Wash Buffer WB. Centrifuge at maximum speed for 1 minute.**
10. **Repeat the washing step once.** Discard the flowthrough from the collection tube. Reinsert the spin column into the collection tube.
11. **Centrifuge at maximum speed for 1 minute** to remove the residual wash buffer .
12. **Transfer the Spin column to a new sterile 1.5ml microcentrifuge tube.** *The resin may pellet against the side of the filter column because of the fixed angle of rotors for most tabletop centrifuges.*
13. **Add 100-200 μ l of nuclease-free water into the spin 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 and/or elute DNA 2 times with 100 μ l of water each time will increase the final yield.*

14. **The purified DNA is ready for downstream application. Or, store the purified DNA at -20°C for later use.**

II. DNA isolation from animal tissues

(See the supplementary information in this manual for the preparation of animal tissue samples)

1. Preparation of tissue lysate

The following methods can be used to disperse and lyse the tissue samples.

1.1 Mechanical homogenization

- A. A: Place the tissue sample in a mini homogenizer. **Add 400 µl of Lysis Buffer LB** to the tube. Homogenize until the tissue is well dispersed. Transfer the homogenized tissue to a sterile 1.5 ml microcentrifuge tube.
- B. **Add 10 µl of Proteinase K. Incubate the tube at 55°C for 30 minutes.**

1.2 Proteinase K digestion

This method can be used to digest directly solid tissue sample and mouse tail.

Cut up to 30 mg of tissue sample , or 1 cm of mouse tail into small pieces, place in a 1.5 ml microcentrifuge tube. **Add 400 µl of lysis buffer LB and 10 µl of Proteinase K. Incubate the tube at 55°C until lysis is complete.** For tissue that is difficult to lyse, lysis overnight (12-16 hours) may be needed. This does not influence the DNA isolation.

2. Proceed as described in steps 3-13 of above

“DNA isolation from cultured cells”

III. DNA isolation from flexible amounts of samples

To isolate genomic DNA from different amounts of samples, such as from less than 10^5 or over 5×10^6 of cultured cells, or over 30mg of animal tissues, solutions and buffers can be adjusted proportionally (Tab.1).

However, a minimum of 100µl of lysis buffer LB and 10µl of DNA binding resin are recommended.

Tab.1 Needed solutions for different amounts of soil samples

sample	5×10^6 of cells, or 30mg of tissues,	Flexible sample
LB(µl)	400	Flexible*
DB(µl)	350	Flexible*
WB(ml)	1.2	Flexible*
Resin(µl)	100	Flexible*
Elution volume(µl)	100-200	200

*:Proportional to the volume for 5×10^6 of cultured cells

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 cell/tissue gDNA Flexspin Kit should be of high purity with the ratio of $OD_{260}/OD_{280} > 1.8$.

Troubleshooting

Problem	Possible Causes	Comments
Incomplete cell lysis	Too large volume of sample	Use less amount of volume or larger volume of solutions
	Incomplete suspension of cell pellet	Vortex or pipet pellet thoroughly
Low yield or no DNA in elute	Ethanol omitted from Wash Buffer	Add ethanol as described
	Poor elution	Add prewarmed EB(>60°C) and incubate for 3 minutes
	Sample is too old	Use fresh sample
	Molecular size of DNA is too big	Vortex or pipet after lysis until the solution is no longer viscous.
RNA contamination	RNAse A omitted	Don't forget to add RNAse A solution
	Insufficient RNase A digestion	increase the incubation time after adding of RNAse A, or add 1 µl of RNAse A to the purified DNA
Spin column is clogged	Too large sample volume or too many cells	Centrifuge until the lysate or solution passes through the column

Spin column is clogged	The centrifuge force is not high enough.	Centrifuge until the lysate or solution passes through the column.
	The lysate is too viscous	Vortex or pipet until the lysate less viscous

Product Use Limitations

XNAPS cell/tissue gDNA 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
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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.