

TECHNICAL HANDBOOK

HMW Genomic DNA Purification Kit

Catalog Number P4001A
P4001B

For purification of **H**igh **M**olecular **W**eight
genomic DNA from
Animal whole blood
Cultured cells
Animal tissues
Plant tissues
Bacteria
Yeast
Fungi

Version 2009

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Kit Contents

Catalog Number	P4001A	P4001B
Kit Size	100 preps	200 preps
Buffer RL	100	200
Lysis Buffer LB	35 ml	70 ml
Buffer SN	15 ml	30 ml
Buffer TE	20 ml	20 ml
RNase A solution	0.5 ml	0.5 ml x 2
Handbook	1	1

*P4001A/P4001B contain all the reagents required for 100/200 preps.

Storage Conditions

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 HMW genomic DNA purification kits are tested on a lot-to-lot basis by purification of genomic DNA from 2×10^6 of cultured cells. 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 HMW genomic DNA purification 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

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Introduction

HMW genomic DNA purification kit provides a simple, efficient and reliable method for isolating genomic DNA with high molecular weight from varieties of organism, such as blood, animal cells/tissues, plant tissues, bacteria, yeast. Although the kit was designed for processing from small volume of starting material, it is easy to scale up to larger volume of samples. After complete lysis of starting material, cellular debris and proteins are removed by selective precipitation and centrifugation. Genomic DNAs with molecular weight up to 150kb are precipitated by ethanol. The procedure provided by the kit eliminates many disadvantages, such as time-consuming methods, 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, real-time PCR, enzymatic digestion, blotting, and hybridizations.

Key Features

1. **Easy processing:** only 3 steps(lysis-protein removal- DNA precipitation) are needed for most samples.
2. **The molecular weight of purified DNA is 100-150kb.**
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/chloroform extraction, no toxic chaotropic salts.
5. **No column or resin is needed.**
6. **Extracted DNA is ready for downstream applications.**

About the amount of starting material

To obtain optimal DNA yield and purity, please refer to Tab. 1 for recommended amount of starting material. For larger volume of samples, adjustment of reagents proportionally will be needed accordingly .

Tab.1 XNAPS Minispin Column Specifications

DNA size purified	100-150 kb
Maximum amount of starting material	
● Whole blood	0.3 ml
● Animal cells	3x 10 ⁶
● Animal tissues	30 mg
● Plant tissues	50 mg
● Bacteria	1 x 10 ⁹
● Yeast	1 x 10 ⁸
● Filamentous fungi	50 mg

Protocols

Materials to be supplied by the user

Microcentrifuge capable of 14000 x g
60°C water bath or heating block
Ethanol(>95%), room temperature
70% ethanol, room temperature
Sterile 1.5ml microcentrifuge tubes
Liquid nitrogen (optional, for tissue lysis)
Mortar and pestle, or tissue homogenizer(optional, for tissue lysis)

I. Lysis of starting material

I.I Lysis of white blood cells from whole blood

Pre-removal of red blood cells and plasma from whole blood will improve the yield and purity of purified genomic DNA. Whole blood treated with EDTA, citrate, or heparin can be used either fresh or frozen. Yield and purity of the purified DNA depend on storage conditions of the blood. Fresher samples yield better results. The following method can separate white blood cells from other components of whole blood.

1. Transfer up to 300 µl of whole blood into a 1.5 ml microcentrifuge tube. *There is no need to complement solution if the starting volume is less than 300 µl.*
2. Add 1 ml of buffer RL. Mix thoroughly by inverting the tube for 4-5 times.
3. Incubate the mixture at room temperature for 10 minutes.
4. Centrifuge at maximum speed for 30 seconds. Decant the supernatant as much as possible.
5. Pulse-vortex the tube for 5-10 seconds to resuspend the pellet in the residual liquid.
6. Add 350 µl of lysis buffer LB to the tube. Completely resuspend the cells by pipetting or pulse-vortexing. *The solution should become clear and very viscous immediately.*
7. Add 5 µl of RNase A solution. Mix thoroughly by vortexing briefly. Incubate the mixture at 37°C for 15-30 minutes.

Proceed directly to II. Isolation of genomic DNA.

I.II Lysis of cultured cells

I.II.A Adherent cells

The following two methods can be used to harvest and lyse cultured adherent cells.

Method 1: trypsin-EDTA treatment

1. Wash the cells with sterile 1x PBS. After removing the PBS, add 1 x trypsin-EDTA(e.g. 1 ml for 100mm plate) and rock the plate to distribute the trypsin solution to cover the cells evenly. Incubate the plate at 37°C for 1-2 minutes.
2. Once the cells are detached, transfer the cells into a new 1.5 ml tube. Centrifuge at top speed for 30 seconds to pellet the cells.
3. Decant the supernatant. Pulse-vortex the tube for 5-10 seconds to resuspend the pellet in the residual liquid.
4. Add 350 µl of lysis buffer LB. Pipett up and down to resuspend the pellet completely. *The cell suspension almost becomes clear and viscous simultaneously with pipetting.*
5. Add 5 µl of RNase A solution. Mix thoroughly by vortexing briefly. Incubate the mixture at 37°C for 30 minutes.

Proceed directly to II. Isolation of genomic DNA.

Method 2: direct lysis of adherent cells

1. Wash the cells with sterile 1x PBS. Remove the PBS completely.
2. Add 350 µl of lysis buffer LB directly into the plate to cover the cells by rocking. Scrape or pipett the cells into the lysis buffer. *The cell suspension almost becomes clear simultaneously with pipetting.*
3. Transfer the lysate into a new 1.5 ml tube.

4. Add 5 µl of RNase A solution. Mix thoroughly by vortexing briefly. Incubate the mixture at 37°C for 15-30 minutes.

Proceed directly to II. Isolation of genomic DNA.

I.II.B Suspension cells

1. Transfer cell suspension into a centrifuge tube. Centrifuge to harvest the cells.
2. Decant the supernatant completely.
3. Add 1 ml of sterile 1x PBS. Resuspend the cells by gently pipetting .
4. Centrifuge for 30 seconds. Decant the supernatant.
5. Add 350 µl of lysis buffer LB. Pipett up and down to resuspend the pellet completely. *The cell suspension almost becomes clear and viscous simultaneously with pipetting.*
6. Add 5 µl of RNase A solution. Mix thoroughly by vortexing briefly. Incubate the mixture at 37°C for 30 minutes.

Proceed directly to II. Isolation of genomic DNA.

I.III Lysis of animal tissues

Up to 30 mg of animal tissue can be used per extraction. The following two methods can be used to homogenize and lyse tissue samples.

Method 1: Liquid nitrogen freeze and grind

1. Flash freeze the tissue sample in liquid nitrogen. Transfer the sample into a mortar with some liquid nitrogen covering the sample. Grind the sample into a fine powder using a pestle.
2. Allow the liquid nitrogen to evaporate while the sample is still frozen.

3. Add 350 µl of lysis buffer LB into the mortar. Grind until the sample is been homogenized completely.
4. Transfer the lysate into a sterile 1.5 ml tube. Incubate the tube at 60°C for 10 minutes to permit complete dissociation of nucleoprotein complexes.
5. Add 5 µl of RNase A solution. Mix thoroughly by vortexing briefly. Incubate the mixture at 37°C for 30 minutes.

Proceed directly to II. Isolation of genomic DNA.

Method 2: Homogenization by homogenizer

1. Cut the tissue into small pieces. Put the sample into appropriate tube or glass Teflon.
2. Add 350 µl of lysis buffer LB. Homogenize manually or by power homogenizer (such as Polytron) . until the tissue is well dispersed.
3. Transfer the lysate into a sterile 1.5 ml tube. Incubate the tube at 60°C for 15 minutes to permit complete dissociation of nucleoprotein complexes.
4. Add 5 µl of RNase A solution. Mix thoroughly by vortexing briefly. Incubate the mixture at 37°C for 30 minutes.

Proceed directly to II. Isolation of genomic DNA.

Method 3: Proteinase K digestion

Animal tissues, including mouse tail, can be lysed completely by Proteinase K digestion. Lysis buffer LB is highly compatible with the activity of Proteinase K.

1. Cut the sample tissue into small pieces. Put the sample into a 1.5 ml tube
2. Add 350 µl of lysis buffer LB and 10 µl of Proteinase K solution (20mg/ml, supplied by user).
3. Incubate the tube at 55 - 60°C until lysis is complete (3 hours may be enough for the most kinds of tissues) .

For tissue that is difficult to lyse, lysis overnight(12-16 hours) may be needed. This does not influence the DNA isolation.

- Cool the tube to room temperature. Add 5 µl of RNase A solution. Mix thoroughly by vortexing briefly. Incubate the mixture at room temperature for 30 minutes.

Proceed directly to II. Isolation of genomic DNA.

I.IV Lysis of bacteria

- Transfer 1 ml of bacterial culture into a new 1.5 ml centrifuge tube. Harvest the bacteria by centrifugation in a table centrifuge at top speed for 1 minute. Decant the supernatant completely.
- Resuspend the pellet thoroughly in 350 µl of lysis buffer LB by vortexing or pipetting up and down.
- Incubate at 60°C for 15 minutes until the solution appears clear.
- Add 5 µl of RNase A solution. Mix thoroughly by vortexing briefly. Incubate the mixture at 37°C for 30 minutes.

Proceed directly to II. Isolation of genomic DNA.

I.V Lysis of yeast

- Transfer 1 ml of yeast culture into a new 1.5 ml centrifuge tube. Harvest the yeast cells by centrifugation in a table centrifuge at maximum speed for 1 minute. Decant the supernatant completely.
- Resuspend the pellet in 100 µl of the following buffer (provided by user):

10mM Tris-HCl, pH 7.5
0.1 M EDTA(pH8.0)
1 M sorbitol
0.1% β-mercaptoethanol
100 units of Lyticase

- Incubate at 30°C for 15-30 minutes until the solution appears clear.
- Add 250 µl of lysis buffer LB. Mix by pipetting or vortexing. Incubate at 60°C for 10 minutes to permit complete dissociation of nucleoprotein complexes.
- Add 5 µl of RNase A solution. Mix thoroughly by vortexing briefly. Incubate the mixture at 37°C for 30 minutes.

Proceed directly to II. Isolation of genomic DNA.

I.VI Lysis of plant tissues

- Flash freeze up to 50 mg of plant tissue in liquid nitrogen. Transfer the sample into a mortar with some liquid nitrogen covering the sample. Grind the sample into a fine powder using a pestle.
- Allow the liquid nitrogen to evaporate while the sample is still frozen.
- Add 350 µl of lysis buffer LB into the mortar. Grind until the sample is been homogenized completely.
- Transfer the lysate into a sterile 1.5 ml tube. Incubate the tube at 60°C for 10 minutes to permit complete dissociation of nucleoprotein complexes.
- Add 5 µl of RNase A solution. Mix thoroughly by vortexing briefly. Incubate the mixture at 37°C for 30 minutes.

Proceed directly to II. Isolation of genomic DNA.

I.VII Lysis of fungi tissue

- Flash freeze up to 50 mg of fungi tissue in liquid nitrogen. Transfer the sample into a mortar with some liquid nitrogen covering the sample. Grind the sample into a fine powder using a pestle.

2. Allow the liquid nitrogen to evaporate while the sample is still frozen.
3. Add 350 µl of lysis buffer LB into the mortar. Grind until the sample is been homogenized completely.
4. Transfer the lysate into a sterile 1.5 ml tube. Incubate the tube at 60°C for 10 minutes.
5. Add 5 µl of RNase A solution. Mix thoroughly by vortexing briefly. Incubate the mixture at 37°C for 30 minutes.

Proceed directly to II. Isolation of genomic DNA.

II. Isolation of genomic DNA

1. Add 150 µl of buffer SN into the tube containing the lysate from above steps. Close the tube and mix thoroughly by vortexing for 10 seconds.
The solution should become cloudy with white precipitates.
2. Incubate the tube on ice for 5 minutes.
3. Centrifuge at maximum speed for 3 minutes. *Clear supernatant should be formed.* Transfer the supernatant into a new tube.
4. Add 1 ml of ethanol into the tube. Invert the tube 4-5 times to mix the solution thoroughly. *Half-transparent, thread-like DNA precipitate will be visible after mixing.*
5. Centrifuge at maximum speed for 2 minutes at room temperature. *A small pellet can be seen on the inner wall. Decant the supernatant carefully.*
6. Add 1 ml of 70% ethanol, Gently invert the tube 4-5 times. Decant the supernatant carefully.
7. Repeat the wash step(step 6) 2 times.
8. Centrifuge the tube briefly. Remove the residual ethanol by pipetting.

9. Stand the tube with cap open for 15 minutes at room temperature (20-25°C). *Sometimes, the DNA pellet may be on the inner wall of the tube. Mark the position of the pellet on the outer wall of the tube.*
10. Add 100 µl of buffer TE. Resuspend the pellet by Pipetting gently up and down. Incubate the tube at 60°C for 1-2 hours to dissolve the DNA, or at room temperature overnight.
To avoid shearing of the purified DNA, resuspend the DNA pellet with pipet tip cut off at the tip so that there is a large opening.
11. The DNA is ready for use. Or, store the purified RNA at 4°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 cell/tissue gDNA Minispin Kit should be of high purity with the ratio of OD_{260}/OD_{280} between 1.7-1.9.

Supplementary Information

I. Preparation of cultured cells

1. For suspension cultures, cells are pelleted by centrifugation. Pellets are washed twice with PBS. Cell pellets can be used immediately or frozen for later use.
2. For adherent cultures, cell monolayer is washed twice with PBS. After the second wash, a small volume of PBS is added to the plates (1 ml for each 100-mm plate), and cells are scraped from the plates. The scraped cells are transferred to eppendorf tubes and pelleted by centrifugation.

II. Preparation of animal tissue samples.

For some types of tissues, such as brain, breast and testis that have higher fat contents, prior removing of lipid may improve the performance of DNA isolation.

To do this, tissue is cut into small pieces and homogenized in PBS until no visible chunks of tissue. Centrifuge the sample at 13000 x g for 20 seconds. Remove the supernatant and lipid as much as possible. Wash the pellet twice by adding 0.5 ml of PBS and pipeting up and down until the pellet is resuspended. Centrifuge at 13000 x g for 20 seconds. After discard the supernatant, the pellet is ready for lysis by adding 0.5 ml of lysis buffer LB.

III. Enhancement of PCR performance

BSA allows positive amplifications in downstream PCR. Using DNA purified from highly contaminated starting materials as template, adding BSA to PCR mixtures to a final concentration of 0.1-1.0 µg/µl can enhance the downstream PCR performance effectively.

IV. Composition of buffers

10 x TE buffer(pH8.0) 100 mM Tris-Cl(pH8.0)
10 mM EDTA(pH8.0)

1xPBS(Phosphate Buffered Saline)

8 g NaCl
0.2 g KCl
1.44 g Na₂HPO₄
0.24 g KH₂PO₄

in 800 ml of distilled H₂O.

Adjust the pH to 7.4 with HCl. Add H₂O to 1 liter. Sterilize by autoclave.

1 x Trypsin-EDTA solution

0.05% trypsin (w/v)
0.53 mM EDTA
Dissolve in 1 x PBS

Troubleshooting

Problem	Possible Causes	Solutions
Low yield of genomic DNA	too large volume or amount of starting material	Use less amount of starting material, such as recommended in the manual.
	Incomplete cell lysis because of incomplete suspension of cell pellet	Vortex or pipet pellet thoroughly until no clumps of cells are visible.
	Poor dissolving of pellet DNA	Incubate at 60°C for 3 hours with tapping the tube periodically.
	Sample is too old	Use fresh sample.
	The DNA pellet was lost during precipitation and washing	Remove the supernatant carefully, ensure the DNA pellet is still in the tube at every step.
Degraded genomic DNA	The starting sample is too old	Use fresh sample.
	Aggressive vortexing or pipetting	Vortexing in less than 30 seconds. Using tips with wide mouth.

Protein contamination ($OD_{260}/OD_{280} < 1.8$)	Incomplete precipitation of protein after adding of buffer SN	Vortexing to mix the solution thoroughly after adding of buffer SN Incubate the tube on ice for 5-10 minutes after adding of buffer SN
	Too large amount of starting material	Use less amount of starting material Add Proteinase K to 200µg/ml (final concentration) in the step of lysis and incubate at 55-60°C for 30 minutes.
RNA contamination	RNAse A digestion omitted	Ensure RNAse A solution is added.
	Insufficient RNase A digestion	increase the incubation time after adding of RNAse A solution.

Product Use Limitations

Express HMW Genomic DNA purification 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.

Notes:

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
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