

TECHNICAL HANDBOOK

XNAPS Plasmid FlexSpin Kit

Catalog Number P1004

For purification of plasmid and cosmid in
miniprep scale
midiprep scale
maxiprep scale

Version 2007

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Kit contents

Kit Catalog Number	P1004
Kit Size*	100 minipreps
Cell Suspension Buffer S1	30 ml
Cell Lysis Buffer S2	30 ml
Neutralization Buffer S3	50 ml
Column Wash Buffer WB	24 ml x 2
DNA Binding Resin	10 ml
Nuclease-free water	20ml
RNase A solution	0.2 ml
Mini Spin Columns	100
Collection Tubes	100
Manual	1

*P1004 contains all the reagents required for 100 minipreps, or 25 midipreps, or 10 maxipreps.

Storage Conditions

Cell Suspension Buffer S1 should be stored at 2-8°C after addition of RNase A. 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 plasmid

flexspin kits are tested on a lot-to-lot basis by purification of plasmid DNA with various sizes(3-17 kb). The yield and purity of purified Plasmid DNA is checked by agarose gel electrophoresis, spectrophotometrical analysis and restriction endonuclease digestion.

Safety Precautions

Although no toxic reagents are contained in XNAPS plasmid purification system, 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 Biolab products from our website.

Contacting information:

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Introduction

The unique and innovative XNAPS plasmid flexspin purification system provides a highly flexible, fast, simple, and efficient method for isolating plasmid DNA from mini (1 ml) to maxi (150 ml) volumes of bacterial culture. The system combines the rapidity and simplicity of spin column method with the flexibility of batch processing for plasmid DNA purification. The novel procedure provided by the kit eliminates many disadvantages, such as time-consuming, toxic components, low yield, from other commercial kits.

The purified plasmid DNA is immediately ready to use in various downstream applications, such as PCR, restriction enzyme digestion, labeling, sequencing, transformation, regular transfection, and *in vitro* transcription/translation.

Key Features

1. **Rapidity:** Depending on the culture volume, all the processing steps can be completed within 8-20 minutes.
2. **High flexibility:** The kit can be used efficiently from small culture volume (1-5 ml) to midi volume (50 ml), even to maxi volume(150 ml).
3. **High yield and purity:** Up to 30-500 µg of high-copy plasmid DNA with an ratio of OD₂₆₀/OD₂₈₀ between 1.8-2.0 can be extracted from 3-150 ml bacterial culture.
4. **Safety for handling, shipping and storage:** No phenol extraction, no ethanol precipitation, no toxic chaotropic salts are necessary.
5. The kit contains enough reagents for 100 minipreps, or 25 midipreps, or 10 maxipreps.

Tab.1 Required volumes of buffers and solutions for various starting cultures (ml)

	Mini	Midi	Maxi
Culture volume (ml)	1-5	Up to 50	Up to 150
Buffer S1 (ml)	0.25	1.2	3
Buffer S2 (ml)	0.25	1.2	3
Buffer S3 (ml)	0.35	2	5
Binding Resin (ml)	0.1	0.4	1
Buffer WB (ml)	1.4	17	18
Elution (ml)	0.1	0.2-0.4	0.4-0.6

Protocols

Note: *The principle of XNAPS plasmid purification system is totally different from the methods of other suppliers. Do not exchange or replace the components of XNAPS plasmid purification kit with components from any other suppliers.*

Materials to be supplied by the user

LB agar plates containing antibiotic
 LB medium containing antibiotic
 Ethanol(>95%)
 Microcentrifuge capable of 14000 x g
 Centrifuge capable of 5000 x g (for midi and maxipreps)
 Sterile 1.5ml microcentrifuge tubes
 Sterile 15 or 30 ml centrifuge tubes (for midi and maxipreps)

Prior to starting:

1. Briefly spin the provided RNase A solution and add it to Buffer S1. Store the S1 containing RNase A at 2-8°C
2. Add 96 ml of ethanol(>95%) to each column wash buffer WB.
3. If buffers in the kit become cloudy or precipitated, heat the bottle containing the buffer up to 60 °C until the solution becomes clear.

I. Preparation of Bacterial Culture

I.A. For minipreps

1. Inoculate a single colony from a fresh LB agar plate directly to 1-5ml of LB medium (containing appropriate antibiotic). Incubate the culture overnight(12-16 hr) at 37°C with vigorous shaking(>200rpm).
Longer incubation is not recommended because plasmid yield may begin to decrease due to cell death and lysis within the aged culture.
2. Harvest the cells by centrifugation at 10000 x g for 2 minutes at room temperature. Pour off the supernatant and blot the inverted tube on a paper towel to remove excess media.

I.B. For midipreps

1. Inoculate a single colony from a fresh LB agar plate directly to 1-2 ml of LB medium (containing appropriate antibiotic). Incubate the culture for 6-8 hr at 37°C with vigorous shaking(>200rpm).
2. Inoculate 0.1-0.2 ml of above culture to 50 ml of LB medium(containing appropriate antibiotic). Incubate the culture overnight(12-16 hr) at 37°C with vigorous shaking(>200rpm).
Longer incubation is not recommended because plasmid yield may begin to decrease due to cell death and lysis within the aged culture.
3. Harvest the cells by centrifugation at 10000 x g for 2 minutes at room temperature. Pour off the supernatant and blot the inverted tube on a paper towel to remove excess media.

I.C. For maxipreps

1. Inoculate a single colony from a fresh LB agar plate directly to 1-2 ml of LB medium(containing appropriate antibiotic). Incubate the culture for 6-8 hr at 37°C with vigorous shaking(>200rpm).
2. Inoculate 0.2 ml of above culture to 100-150 ml of LB medium(containing appropriate antibiotic). Incubate the culture overnight(12-16 hr) at 37°C

with vigorous shaking(>200rpm).

Longer incubation is not recommended because plasmid yield may begin to decrease due to cell death and lysis within the aged culture.

3. Harvest the cells by centrifugation at 10000 x g for 2 minutes at room temperature. Pour off the supernatant and blot the inverted tube on a paper towel to remove excess media.

II. Preparation of Bacterial Lysate**II.A. For Miniprep (From 1-5 ml of overnight culture)**

1. Add 250µl of buffer S1. Resuspend the cell pellet completely by vortexing or pipetting.
Complete suspension is essential for thorough lysis of the cells in the next step.
2. Add 250µl of buffer S2, mix gently by inverting the tube 4-5 times. Incubate at room temperature until the cell suspension clears, but do not exceed 5 minutes. *Do not vortex at this and next step, as this will result in shearing of genomic DNA. The lysate should become viscous after addition of buffer S2.*
3. Add 350µl of buffer S3, mix thoroughly by inverting the tube 4-5 times. The solution should become cloudy.

4. Centrifuge at maximum speed in a tabletop centrifuge for 1 minute. *Clear supernatant should be formed, although a few white precipitants may float on the top.* If floating precipitant is a concern, increase centrifuge time to 5-10 minutes.

II.B. For Midiprep (From 50 of overnight culture)

1. Add 1.2 ml of buffer S1. Resuspend the cell pellet by vortexing or pipetting completely. *Complete suspension is essential for thorough lysis of the cells in the next step.*
2. Add 1.2 ml of buffer S2, mix gently by inverting the tube 4-5 times. Incubate at room temperature until the cell suspension clears, but do not exceed 5 minutes. *Do not Vortex at this and next step, as this will result in shearing of genomic DNA. The lysate should become viscous after addition of buffer S2.*
3. Add 2 ml of buffer S3, mix thoroughly by inverting the tube 4-5 times. The solution should become cloudy and less viscous. The fluffy white material contains genomic DNA, proteins, cell debris and SDS.

4. Centrifuge at 13000 x g for 10 minutes at room temperature. *Clear supernatant should be formed, although a few white precipitants may float on the top.*

II.C. For Maxiprep (From 150 of overnight culture)

1. Add 3 ml of buffer S1. Resuspend the cell pellet by vortexing or pipetting completely. *Complete suspension is essential for thorough lysis of the cells in the next step.*
2. Add 3 ml of buffer S2, mix gently by inverting the tube 4-5 times. Incubate at room temperature until the cell suspension clears, but do not exceed 5 minutes. *Do not Vortex at this and next step, as this will result in shearing of genomic DNA. The lysate should become viscous after addition of buffer S2.*
3. Add 5 ml of buffer S3, mix thoroughly by inverting the tube 4-5 times. The solution should become cloudy and less viscous. The fluffy white material contains genomic DNA, proteins, cell debris and SDS.
4. Centrifuge at 13000 x g for 10 minutes at room temperature. *Clear supernatant should be formed, although a few white precipitants may float on the top.*

III. Isolation of Plasmid

III.A. For minipreps

Assemble the plasmid isolation units by inserting one spin column into one 2ml collection tube for each sample.

1. Pour or pipet the clear supernatant from II.A. into the spin column.

Avoid transferring any of the white precipitate with the supernatant into the column.

2. Add 100 µl of DNA binding resin into the clear supernatant from II.A.

Note: Resuspend the resin thoroughly by vortexing or shaking before using. Mix gently by pipetting up and down.

3. Stand at room temperature for 1 minute.

Centrifuge at maximum speed for 1 minute.

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

4. Discard the flowthrough from the collection tube.
5. Add 700µl of wash buffer WB . Centrifuge at maximum speed for 1 minute.
6. Discard the flowthrough from the collection tube. Reinsert the spin column into the collection tube.
7. Repeat the washing (Step 5 and 6) once.

8. Centrifuge at maximum speed for 2 minutes.
9. Transfer the spin column to a new, sterile 1.5ml microcentrifuge tube.
10. Add 100 µl of nuclease-free water into the center of the spin column. The water should be applied to immerse the highest portion of pellet resin. Stand at room temperature for 1 minute. Centrifuge at maximum speed for 1 minute. *For maximum recovery of plasmid, resuspend the resin into the water by pipetting.*
11. Store the plasmid at -20°C. To store the plasmid at 4°C , add 11µl of 10xTE buffer to the 100µl of eluted plasmid solution.

III.B. For midipreps

Assemble the plasmid isolation units by inserting one spin column into one 2ml collection tube for each sample.

Each midipreps needs 2 sets of spin columns.

1. Transfer carefully the clear supernatant from II.B. into a sterile centrifuge tube.

Avoid transferring any of the white precipitate with the supernatant into the column.

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

2. Add 0.4 ml of DNA binding resin into the

- supernatant. Mix gently by inverting the tube 4-5 times. Stand at room temperature for 1 minute.
3. Centrifuge at 6000 x g for 1 minute. Decant the supernatant from the tube.
 4. Resuspend the pellet with 4 ml of wash buffer WB by pipetting up and down or vortexing briefly. Centrifuge at 6000 x g for 1 min. Decant the supernatant from the tube.
 5. Repeat the washing with another 4 ml of WB.
 6. Resuspend the resin in 1 ml of WB. Transfer the suspension into the 2 spin columns (about 0.6 ml each).
 7. Centrifuge at maximum speed in a microcentrifuge for 1 minute. Discard the flowthrough from the collection tube.
The resin may pellet against the side of the filter column because of the fixed angle of rotors for most tabletop centrifuges.
 8. Reinsert the spin column into the collection tube. Centrifuge at maximum speed for 2 minutes.
 9. Transfer the spin column to a new, sterile 1.5ml microcentrifuge tube. Add 100-200 µl of nuclease-free water into the center of each spin column. The water should be applied to immerse

the highest portion of pellet resin. Stand at room temperature for 1 minute. Centrifuge at maximum speed for 2 minutes.

Tips: *Resuspending the resin with the nuclease-free water by pipetting can increase the plasmid yield.*

10. Combine the duplicate plasmid in one tube and store at -20°C. To store the plasmid at 4°C, add 11 µl of 10xTE buffer to the 100 µl of eluted plasmid solution.

III.C. For maxipreps

Assemble the plasmid isolation units by inserting one spin column into one 2ml collection tube for each sample.

Each maxipreps needs 2 sets of spin columns.

1. Transfer carefully the clear supernatant from II.C. into a sterile centrifuge tube.

Avoid transferring any of the white precipitate with the supernatant into the column.

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

2. Add 1 ml of DNA binding resin into the supernatant. Mix gently by inverting the tube 4-5 times. Stand at room temperature for 1 minute.
3. Centrifuge at 6000 x g for 1 minute. Decant the supernatant from the tube.

4. Resuspend the pellet with 8 ml of wash buffer WB by pipetting up and down or vortexing briefly. Centrifuge at 6000 x g for 1 min. Decant the supernatant from the tube.
5. Repeat the washing with another 8 ml of WB.
6. Resuspend the resin in 1 ml of WB. Transfer the suspension into the 2 spin columns (about 0.75 ml each).
7. Centrifuge at maximum speed in a microcentrifuge for 1 minute. Discard the flowthrough from the Collection tube.
The resin may pellet against the side of the filter column because of the fixed angle of rotors for most tabletop centrifuges.
8. Reinsert the spin column into the collection tube. Centrifuge at maximum speed for 2 minutes.
9. Transfer the spin column to a new, sterile 1.5ml microcentrifuge tube. Add 200-300 µl of nuclease-free water into the center of each spin column. The water should be applied to immerse the highest portion of pellet resin. Stand at room temperature for 1 minute. Centrifuge at maximum speed for 2 minutes.

Tips: Resuspending the resin with the nuclease-free water by pipetting can increase the plasmid yield.

10. Combine the duplicate plasmid in one tube and store at -20°C. To store the plasmid at 4°C, add 11µl of 10xTE buffer to the 100µl of eluted plasmid solution.

Yield and Purity Examination

Both spectrophotometrical analysis and agarose gel electrophoresis are recommended for the yield and purity determination of the purified plasmid DNA. To determine the concentration of plasmid 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 plasmid DNA can be calculated by multiplying the concentration by the volume of DNA solution.

The plasmid DNA purified by XNAPS plasmid flexspin kit should be of superior purity with the ratio of OD_{260}/OD_{280} between 1.8-2.0.

Supplementary Information

Based on the Renogen proprietary nucleic acid purification platform, XNAPS plasmid purification system is designed to extract and purify plasmid, cosmid, and single- or double-stranded M13 DNA from *E. coli* cultures. The method is optimized for high yield and ultra-purity of plasmid DNA extraction. Yet a number of factors, such as different *E. coli* strains, plasmid copy number, density of bacterial culture, components of medium, still affect the outcome and performance of the processing.

1. *E. coli* strains

While reports based on a few other brand kits showed that endogenous endonuclease I from some EndA⁺ *E. coli* strains may cause some kind of degradation of plasmid DNA in the processing, XNAPS plasmid purification system demonstrates an extraordinary performance for both EndA⁺ and EndA⁻ strains tested. However, if the quality of purified plasmid DNA is not as expected, or the endogenous endonuclease I is a concern, we recommend changing to a EndA⁻ strain (Tab.2).

Tab.2 Common EndA⁻ and EndA⁺ *E. coli* strains

EndA ⁻	EndA ⁺
DH1	BL21(DE3)
DH20	HB101
DH21	JM83
DH5α	JM101
JM103	JM110
JM105	TB1
JM106	TG1
JM107	
JM108	
JM109	
TOP10	
XL1-Blue	

2. Plasmid copy number

Large variation of copy number of various plasmids (Tab.3), because of the capability of its replication origin, influences the yield of purification. For plasmid with high copy number, it is easy to extract 5-10 µg from 1 ml of overnight culture. For plasmid with low copy number, increasing culture volume or amplifying by chloramphenicol may be considered.

Tab.3 Copy numbers of some plasmids and their derivatives

plasmid	Origin of replication	Copy number
pUC series	pMB1	500-700
pBluescript series	ColE1	300-500
pGEM series	pMB1	300-400
pBR322	pMB1	15-20
pSC101	pSC101	~5

3. Density of bacterial culture

Under the recommended condition, bacterial culture may grow to an OD₆₀₀ of 1.0-3.0 after 12-16 hours, equals to 1.5 to 3.0 x 10⁹ bacteria/ml. At this point, the ratio of plasmid DNA to RNA is high, yet with no obvious degradation due to overaging of the culture. Optimal culture densities and sample volumes may vary depending on the host strain and can be adjusted accordingly. Nevertheless, too higher density may overload the purification system and clog the binding column, which will decrease both the yield and purity of plasmid.

4. Type of medium

Standard LB (Luria-Bertani) medium is recommended for use with XNAPS plasmid purification system. Although different LB recipes also can be used, it is found that *E. coli* growing in standard LB (containing 10 g of NaCl per liter, see Composition of culture media and solutions) harbours the highest number of plasmids. Rich media, such as 2 x YT and TB (Terrific Broth), may be used. However, *E. coli* growing overnight in them lead to a higher percentage of dead or starving bacteria. This may result in degraded or contaminated plasmid. Thus, when using rich media, shorten growth times within 12 hours.

5. Composition of culture media and solutions

1). Standard LB medium

per liter	Tryptone	10g
	Yeast extract	5g
	NaCl	10g
	pH	7.0

2). LB medium plate

per liter	Tryptone	10g
	Yeast extract	5g
	NaCl	10g
	Bacto agar	15g
	pH	7.0

3). 10x TE buffer (pH8.0)

100mM Tris-HCl (pH8.0)
10mM EDTA (pH8.0)

4). Stock solutions of antibiotics (store at -20°C)

Antibiotic	Stock solution	Solvent	Storage	Working concentration
Ampicillin	100mg/ml	H ₂ O	-20°C	100 µg/ml
Kanamycin	25mg/ml	H ₂ O	-20°C	50 µg/ml
Chloramphenicol	34 mg/ml	Ethanol	-20°C	170 µg/ml
Tetracycline	5 mg/ml	Ethanol	-20°C	50 µg/ml

Troubleshooting

Problem	Possible Causes	Comments
Incomplete cell lysis	Too many cells in overnight culture	Use recommend medium and culture volume
	Incomplete suspension of cell pellet	Vortex or pipet cell pellet thoroughly

Low yield or no plasmid DNA in elute	Ethanol omitted from Wash Buffer	Add ethanol as described
	Poor elution	Add Elution Buffer on the center of spin column, incubate for 1 minute
	Cell colony or medium is too old	Use fresh colony and medium with appropriate antibiotics
Genomic DNA contamination	Low copy number plasmid	Increase amount of culture to 10-15ml, lyse as 3 samples, but use the same column to binding plasmid.
	Cell overgrown	Reduce the incubation time
	Mixed too vigorously in the steps of lysis and neutralization	Do not vortex. Mix gently by inverting the tube 4-5 times
RNA contamination	Over-lysis in step 2	Do not exceed 5 minutes
	RNase A omitted from Cell Suspension Buffer	Ensure RNase A added to buffer S1
Spin column is clogged	Insufficient RNase A digestion	Reduce cells, or add additional RNase A to S1
	Too large sample volume or too many cells	Ensure that no more than 200 µL of blood is applied to the column.
	The centrifuge force or time is not high enough.	increase the g-force and/or centrifuge for a longer period of time until the lysate or solution passes through the column.

Product Use Limitations

XNAPS Plasmid 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.

References

1. Vogelstein B. et al. Preparative and analytical purification of DNA from agarose. PNAS 76, 615-619, 1979
2. Birnboim HC et al. A rapid alkaline lysis procedure for screening recombinant plasmid DNA. Nucl. Acids Res. 7, 1513-1522, 1979
3. Sambrook J. et al. Molecular Cloning: a laboratory manual. 2nd edition. Cold Spring harbor Laboratory Press. 1989
4. Birnboim HC et al. A rapid alkaline extraction method for the isolation of plasmid DNA. Methods Enzymol. 100, 243-255, 1983

Product Selection Guide

	Sample source	Sample quantity	Kit name and description	Cat. No.*
Plasmid	Culture medium	Mini(1-5 ml)	XNAPS plasmid minispin kit	NP1001A/B
		Flexible (1-150 ml)	XNAPS plasmid flexspin kit(100)	NP1004
Genomic DNA	Blood Buffy coat	Up to 0.3ml	XNAPS blood gDNA minispin kit	NP1011A/B
		10µl-10ml	XNAPS blood gDNA flexspin kit(100)	NP1014
	Cell/tissue	2x10 ⁶ cells or 30mg tissue	XNAPS cell/tissue gDNA minispin kit	NP1016A/B
		10 ³ -10 ⁷ cells or 1-100mg tissue	XNAPS cell/tissue gDNA flexspin kit(100)	NP1017
	Stool	100mg	XNAPS stool DNA minispin kit	NP1022A/B
		Flexible	XNAPS stool DNA flexspin kit(100)	NP1023
	swabs	one	XNAPS swab DNA minispin kit(100)	NP1024
	Body fluid, plasma, serum	Up to 0.25ml	XNAPS body fluid DNA minispin kit	NP1021A/B
Up to 10ml		XNAPS body fluid DNA Flexspin kit(100)	NP1026	

Genomic DNA	Plant tissue	100mg	XNAPS plant DNA minispin kit(100)	NP1025
	soil	Up to 250mg	XNAPS soil DNA minispin kit	NP1034A/B
		Flexible	XNAPS RNA minispin kit(100)	NP1035
	Micro amount sample	Micro volume	XNAPS MicroDNA flexbond kit(100)	NP1038
Viral nucleic acid	Liquid sample	<0.25ml	XNAPS viral nucleic acid minispin kit	NP1032A/B
		Flexible	XNAPS viral nucleic acid flexbond kit(100)	NP1033
Total RNA	Animal cell, tissue	2x10 ⁶ cells, 30mg tissue	XNAPS RNA Minispin Kit	NP2001A/B
Others	Agarose gel, solution	Flexible	FastBack DNA minispin kit	NP3001A/B

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

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