

# TECHNICAL HANDBOOK

## XNAPS RNA Flexspin Kit

Catalog Number P2001A  
P2001B

For purification of total RNA from  
Animal whole blood  
Cultured cells  
Animal tissues  
Plant tissues  
Bacteria  
Yeast  
Fungi

Version 2009

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**Kit Contents**

<b>Catalog Number</b>	<b>P2001A</b>	<b>P2001B</b>
Kit Size	50 preps	100 preps
Lysis buffer LS	30 ml	60 ml
Denaturation buffer DS	20 ml	40 ml
Wash buffer WB	12 ml	24 ml
RNA 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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**Storage Conditions**

Store the RNA 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 monitoring and detection of the kit components, the performance of XNAPS RNA flexspin kits are tested on a lot-to-lot basis by purification of total RNA from  $2 \times 10^6$  of cultured cells. The yield and purity of purified RNA 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.

Contacting information:

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## Introduction

XNAPS RNA flexspin purification system provides a fast, simple, and efficient method for isolating total RNA from varieties of organism, such as animal cells/tissues, plant tissues, bacteria, yeast. After chemical lysis of samples, proteins and genomic DNA are firstly removed by selective precipitation and centrifugation. Residual DNA can be further enzymatically digested. High-quality RNA is eluted into nuclease-free water. The novel 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 RNA is immediately ready to use in various downstream applications, such as RT-PCR, cDNA library construction, Northern blotting, and expression array assays.

## Key Features

1. **Rapid and easy processing:** All the processing steps can be completed in 20 minutes.
2. **High yield and purity:** OD<sub>260</sub>/OD<sub>280</sub> of purified RNA is between 1.9-2.1. No DNA contamination.
3. **Efficient isolation of intact RNAs with wide range of sizes** from rRNA to miRNA.
4. **Safety** for handling, shipping and storage: No phenol extraction, no ethanol precipitation, no toxic chaotropic salts.
5. **Extracted RNA is ready for downstream application**

### About the amount of starting material

The amount of starting material plays an important role in RNA extraction. It may influence the processing and the final yield and purity of extracted RNA. To obtain optimal RNA yield and purity, please refer to Tab. 1 for maximum amount of starting material. For samples containing high or low amounts of RNA, adjustment of starting amount may be needed.

**Tab.1 XNAPS Flexspin Column Specifications**

Column binding capacity	100 µg
Maximum loading volume	1000 µl
RNA size purified	All sizes of RNA
Maximum amount of starting material	
● Whole blood	0.3 ml
● Animal cells	$5 \times 10^6$
● Animal tissues	30 mg
● Plant tissues	50 mg
● Bacteria	$1 \times 10^9$
● Yeast	$1 \times 10^8$
● Filamentous fungi	50 mg

### Protocols

*The principle of XNAPS RNA flexspin kit is totally different from the methods of other suppliers. Do not exchange or replace the components of XNAPS RNA 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(>95%)

Sterile 1.5ml microcentrifuge tubes

(optional) Liquid nitrogen

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(optional) Mortar and pestle, or tissue homogenizer

(optional) 70% ethanol(in RNase-free water)

(optional) DNase I solution

**Prior to starting:**

1. Add 48 and 96 ml of ethanol(>95%) to wash buffer WB of P2001A and P2001B, respectively.
2. Preheat a water bath or heating block to 60°C.
3. If solutions from the kit are cloudy or precipitated, heat the bottle containing the buffer to 60 °C until the solution becomes clear.

**Prevention of RNase contamination**

RNases are robust and powerful enzymes that may degrade RNAs at all stages of isolation. However, problems with exogenous RNases can be entirely avoided by vigilant use of prophylactic measures and the prudent application of common sense(For details, see *Molecular Cloning: A Laboratory Manual*). Following hints may help prevent RNase contamination in experiment processing.

1. Creating a RNase-free environment. Set aside items of glassware, batches of plasticware, and buffers that are to be used only for experiments with RNA; Use sterile techniques when handling the reagents for RNA processing.
2. Wear gloves at all times.
3. Treat solutions supplied by the user by 0.1% of diethyl pyrocarbonate(DEPC) for at least 1 hour at 37°C or overnight at room temperature. Autoclave for 15 minutes at 15 psi to remove any trace of DEPC.
4. Use disposable tips and microcentrifuge tubes certified by a reputable manufacturer to be free of RNase.
5. RNase inhibitors may be needed during the isolation of RNA and its downstream applications.

## I. Preparation of clear lysate

### I.I Preparation of lysate from whole blood

Pre-removal of red blood cells and plasma from whole blood will improve the yield and purity of extracted RNA dramatically. Whole blood treated with EDTA, citrate, or heparin can be used either fresh or frozen. Yield and purity of the purified RNA 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 300 µL of whole blood into a 1.5 ml tube.
2. Add 1 ml of red blood cell lysis buffer (Provided by user, See Buffers and Solutions from **Supplementary Information** for composition) . Mix thoroughly by inverting 4-5 times.
3. Incubate at room temperature for 10 minutes.
4. Centrifuge at top speed for 1 minutes. Remove completely the supernatant.
5. Add 400 µL of lysis buffer LS to the pellet. Resuspend the pellet by pipetting or vortexing. Incubate at room temperature for 10 minutes.

### I.II Preparation of lysate from cultured cells

Each column of XNAPS RNA flexspin kit is capable of extracting RNA efficiently from up to  $3 \times 10^6$  cultured cells. The number of cells used, however, may need to be adjusted depending on cell type, function and RNA expression levels at the time of harvest.

#### 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. Decant the supernatant.
3. Add 400 µl of lysis buffer LS. Pipett up and down to resuspend the pellet. *The cell suspension almost becomes clear simultaneously with pipetting.*
4. Incubate the tube at room temperature for 10 minutes.

**Proceed directly to II. Isolation of Total RNA.****Method 2:** direct lysis of adherent cells

1. Wash the cells with sterile 1x PBS. Remove the PBS completely.
2. Add 400 µl of lysis buffer LS 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. Incubate the tube at room temperature for 10 minutes.

**Proceed directly to II. Isolation of Total RNA.****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 400 µl of lysis buffer LS. Pipett up and down to resuspend the pellet. *The cell suspension almost becomes clear simultaneously with pipetting.*
4. Incubate the tube at room temperature for 10 minutes.

**Proceed directly to II. Isolation of Total RNA.****I.III Preparation of lysate from 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 600 µl of lysis buffer LS 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 room temperature for 10 minutes.
5. Centrifuge at top speed for 2 minutes at room temperature.
6. Transfer 400 µl of supernatant into a new sterile 1.5 ml tube.

**Proceed directly to II. Isolation of Total RNA.**

**Method 2: Homogenization by homogenizer**

1. Cut the tissue into small pieces. Put the sample into appropriate tube or glass Teflon.
2. Add 600 µl of lysis buffer LS. Homogenize manually or by power homogenizer (such as Polytron).
3. Transfer the lysate into a sterile 1.5 ml tube. Incubate the tube at room temperature for 10 minutes.
4. Centrifuge at top speed for 2 minutes at room temperature.
5. Transfer 400 µl of clear supernatant into a new sterile 1.5 ml tube.

**Proceed directly to II. Isolation of Total RNA.**

**I.IV Preparation of lysate from bacteria**

1. 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.
2. Resuspend the pellet thoroughly in 400 µl of lysis buffer LS by vortexing or pipetting up and down.
3. Incubate at 60°C for 10-15 minutes until the solution appears clear.

**Proceed directly to II. Isolation of Total RNA.**

**I.V Preparation of lysate from yeast**

1. 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 top speed for 1 minute. Decant the supernatant completely.
2. 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
3. Incubate at 30°C for 15-30 minutes until the solution appears clear.
4. Add 300 µl of lysis buffer LS. Incubate at 60°C for 10.

**Proceed directly to II. Isolation of Total RNA.**

### **I.VI Preparation of lysate from plant tissues**

1. 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.
2. Allow the liquid nitrogen to evaporate while the sample is still frozen.
3. Add 600  $\mu$ l of lysis buffer LS 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. Centrifuge at top speed for 2 minutes at room temperature.
6. Transfer 400  $\mu$ l of supernatant into a new sterile 1.5 ml tube.

**Proceed directly to II. Isolation of Total RNA.**

### **I.VII Preparation of lysate from fungi tissues**

1. 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 600  $\mu$ l of lysis buffer LS 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. Centrifuge at top speed for 2 minutes at room temperature.
6. Transfer 400  $\mu$ l of supernatant into a new sterile 1.5 ml tube.

**Proceed directly to II. Isolation of Total RNA.**

## **II. Isolation of Total RNA**

1. Add 300  $\mu$ l of buffer DS into the tube containing the lysate from above steps. Close the tube and mix thoroughly by inverting the tube 4-5 times or vortexing briefly.

*The solution should become cloudy with white precipitates.*

2. Incubate the tube on ice for 5 minutes.
3. Centrifuge at maximum speed for 5 minutes.
4. Insert a filter column into a collection tube. One set for each sample.

5. Decant gently the clear supernatant into the filter column.
6. Add 100 µl of RNA binding resin. Mix thoroughly by pipetting up and down.

**Tip: Resuspend the RNA binding resin by shaking or vortexing before transferring.**

7. Centrifuge at maximum speed for 1 minute at room temperature. Discard the flowthrough. Re-insert the column into the collection tube.
8. (optional) If removal of trace genomic DNA is needed, “on-column” digestion of DNA can be performed at this point ( see **Supplementary Information** ) .
9. Add 600 µl of wash buffer WB into the centrifuge column. Centrifuge at maximum speed for 1 minute at room temperature. Discard the flowthrough. Re-insert the column into the collection tube.
10. Repeat the wash steps( step 9) once. Discard the flowthrough. Re-insert the column into the collection tube.
11. Centrifuge at maximum speed for 2 minutes.
12. Transfer the column into a new sterile 1.5 ml tube. Add 100-200 µl of nuclease-free water into the center of column. Stand the tube at room temperature for 1 minute.
13. Centrifuge at maximum speed for 1 minute. The RNA is ready for use. Or, store the purified RNA at -70°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 extracted RNA. To determine the concentration of RNA by spectrophotometer, the following formula should be used :

$$[\text{RNA}] (\mu\text{g/ml}) = A_{260} \times 40 \times D,$$

where D is the dilution factor, and 1 absorbance unit of  $A_{260}$  equals 40 µg of single-stranded RNA/ml. The yield of DNA can be calculated by multiplying the concentration by the volume of RNA solution.

The R purified by XNAPS RNA flexspin kit should be of high purity with the ratio of  $OD_{260}/OD_{280}$  between 1.9-2.1.

## Supplementary Information

### I. “on-column” removal of DNA contamination

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All currently available RNA purification methods will inevitably co-extract genomic DNA in their processing, even when it is not visible on an agarose gel. While XNAPS RNA flexspin kit can remove the vast majority of cellular DNA, trace amounts may still remain. In most circumstances, these residual DNA do not interfere with the downstream application of RNA. However, further DNA removal may be necessary for certain RNA applications that are sensitive to very small amount of DNA, such as real-time RT-PCR. The following method of “on-column” removal of DNA contamination can digest trace DNA efficiently in the extracting process. The DNase I and digested DNA are washed away in the next wash steps, only leaving RNA bound on column.

#### **Protocol of “on-column” DNA digestion.**

After the step 5 of II. Isolation of Total RNA, do the following steps instead of performing the first wash step.

1. Preparation of DNase I working solution(provide by user). Dilute RNase-free DNase I with diluting buffer( 25 mM Tris-HCl, pH 7.6, 10 mM MgCl<sub>2</sub>, 2 mM CaCl<sub>2</sub>) to 0.25 Kunitz unit/μl. A 100 μl aliquot is required for each column to be treated.
2. Add 500 μl of 70% ethanol (in RNase-free water) into the column. Centrifuge at top speed for 1 minute at room temperature.
3. Decant the flowthrough. Re-insert the column into the collection tube.
4. Centrifuge at top speed for 1 minute at room temperature.
5. Add 100 μl of DNase I working solution from step 1 into the column. Make sure that the Solution covers all the surface of the membrane.
6. Incubate for 15 minutes at 20-25°C. *There is no need to centrifuge before the next step.*
7. **Proceed directly to Step 7 of II. Isolation of Total RNA.**

## **II. Composition of Buffers and Solutions**

### **10 x PBS**

11.5 g Na<sub>2</sub>HPO<sub>4</sub>  
2.0 g KH<sub>2</sub>PO<sub>4</sub>  
80.0 g NaCl  
2.0 g KCl

Dissolve in 1 liter of sterile, deionized water. The pH of 1xPBS will be 7.4

### **Red blood cell lysis buffer**

10 mM Tris-HCl, pH7.6

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**1 x Trypsin-EDTA solution**

0.05% trypsin (w/v)

0.53 mM EDTA

Dissolve in 1 x PBS

**Troubleshooting**

<b>Problem</b>	<b>Possible Causes</b>	<b>Comments</b>
Low yield or no RNA in elute	Poor lysis	Use enough lysis buffer, do not exceed the recommended amounts of starting materials
		Incubate at 65°C for 10 minutes, or until the suspension is clear
	Sample too old	Use fresh samples
	Ethanol omitted from Wash Buffer	Add ethanol as described
	Low RNA content in samples	Increase the amount of starting material, scale up the extraction
	Poor elution	Use prewarmed water (>60°C) to resuspend the resin and incubate for 2 minutes
RNA degradation	RNase contamination	Exogenous RNase was introduced in the processing. See” <b>Prevention of RNase contamination</b> ” at the begin of this handbook.
	RNA was degraded during sample prep.	Work quickly during sample preparation, especially in the steps of preparing sample lysate.
	Improper storage of starting sample	Use fresh samples, or store samples at <-70°C.
	Improper storage of extracted RNA	store the RNA solution at -70°C.
DNA contamination	Large amounts of starting materials used	Adjust the amounts of starting materials. Do “on-column” DNA digestion as described in <b>Supplementary information</b> .

	Incomplete DNase digestion “on-column”	Check the DNase digestion system. Make sure the DNase solution permeate into the membrane.
Spin Column is clogged	Too large sample volume or too many cells	Reduce the amount of starting material.
		Centrifuge for a longer period of time until the lysate or solution passes through the column
	Incomplete lysis	Use the appropriate amount of lysis buffer
	Centrifuge temperature is too low	Ensure the centrifuge temperature is 20-25°C.

### Product Use Limitations

XNAPS RNA 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.

**XNAPS and related Products**

<b>Application</b>	<b>Sample source</b>	<b>Sample quantity</b>	<b>Kit name and description</b>	<b>Cat. No.*</b>	<b>Price</b>
Plasmid purification	Culture medium	Flexible (1-150 ml)	XNAPS plasmid endofree flexspin kit	NP1003A/B	\$150/270
		Flexible (1-150 ml)	XNAPS plasmid flexspin kit	NP1004A/B	\$60/120
		0.5 ml	XNAPS 96 well plasmid flexspin kit	NP1005A/B	\$120
Genomic DNA purification	Blood Buffy coat	10µl-10ml	XNAPS blood gDNA flexspin kit	NP1014A/B	\$80/150
	Animal cell , tissue, swab	10 <sup>3</sup> -10 <sup>7</sup> cells or 1-100mg tissue	XNAPS cell/tissue gDNA flexspin kit	NP1017A/B	\$80/150
	Stool	Flexible	XNAPS stool DNA flexspin kit	NP1023A/B	\$90/175
	Body fluid, plasma, serum	Up to 10ml	XNAPS body fluid DNA Flexspin kit	NP1026A/B	\$80/150
	Plant tissue	100mg	XNAPS plant DNA Flexspin kit	NP1025A/B	\$90/175
	soil	Flexible	XNAPS soil DNA flexspin kit	NP1035A/B	\$90/175
	Micro amount sample	Micro volume	XNAPS MicroDNA flexbond kit(100)	NP1038	\$175
Total RNA	Animal cell, tissue	2x10 <sup>6</sup> cells, 30mg tissue	XNAPS RNA Flexspin Kit	NP2001A/B	\$150/\$250
Others	Agarose gel, solution	Flexible	FastBack DNA minispin kit	NP3001A/B	\$60/\$120

## Ordering Information

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### **Customers in USA and Canada**

*To place an order, please use any of the following ways:*

<p><b>Phone:</b> 1-866-712-4412(Toll free) Mon.-Fri 8:00am-5:00pm (EST)</p> <p><b>Fax:</b> 1-651-204-9348</p> <p><b>Mail:</b> #310, 2386 East Mall Vancouver, BC, V6T 1Z3 Canada</p>
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### **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:*

<p><b>Phone:</b> 1-651-204-0326 Mon.-Fri 8:00am-5:00pm (EST)</p> <p><b>FAX:</b> 1-651-204-9348</p> <p><b>Mail:</b> #310, 2386 East Mall Vancouver, BC, V6T 1Z3 Canada</p>
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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](http://www.renogenbio.com) for detailed information