

# Hybrid-R™ miRNA

SMALL RNA PURIFICATION HANDBOOK

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We thank you for any comment or advice.

### Contact us at

[www.geneall.com](http://www.geneall.com)

Tel : 82-2-407-0096

Fax : 82-2-407-0779

E-mail(Order/Sales) : [sales@geneall.com](mailto:sales@geneall.com)

E-mail(Tech. Info.) : [tech@geneall.com](mailto:tech@geneall.com)

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This protocol handbook is included in :

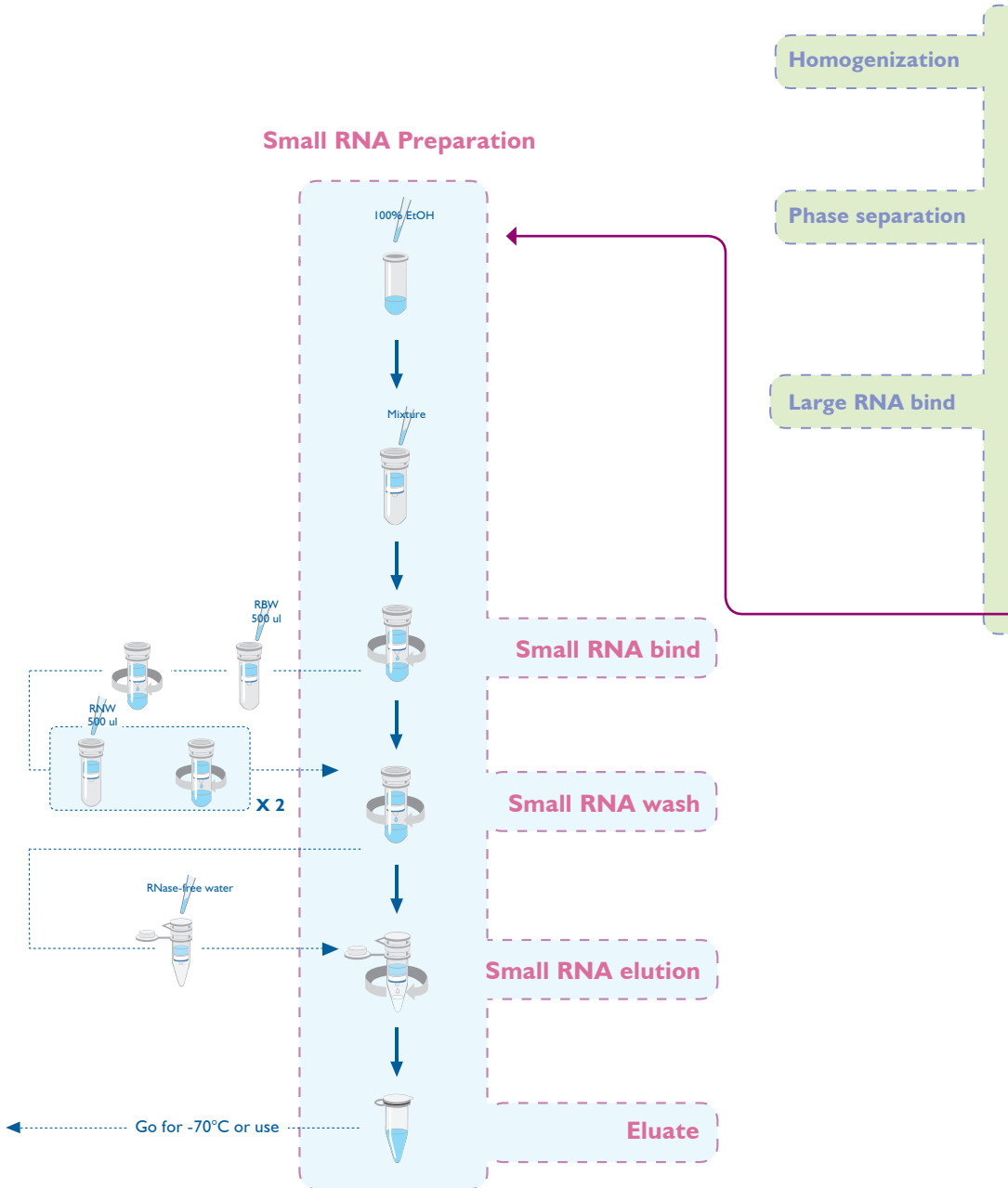
GeneAll® Hybrid-R™ miRNA (325-150)

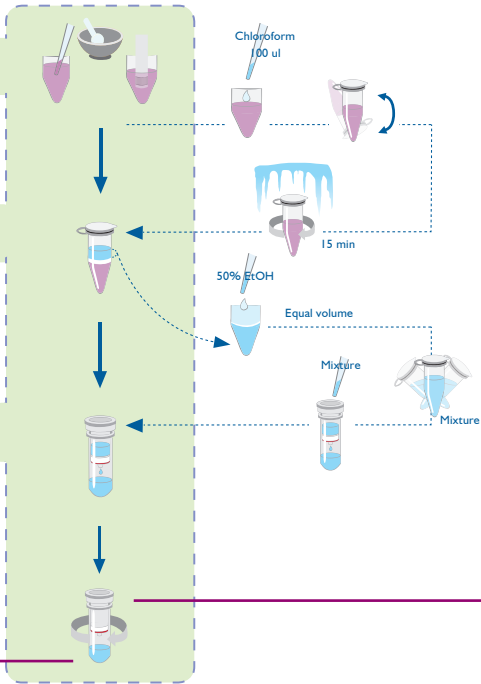
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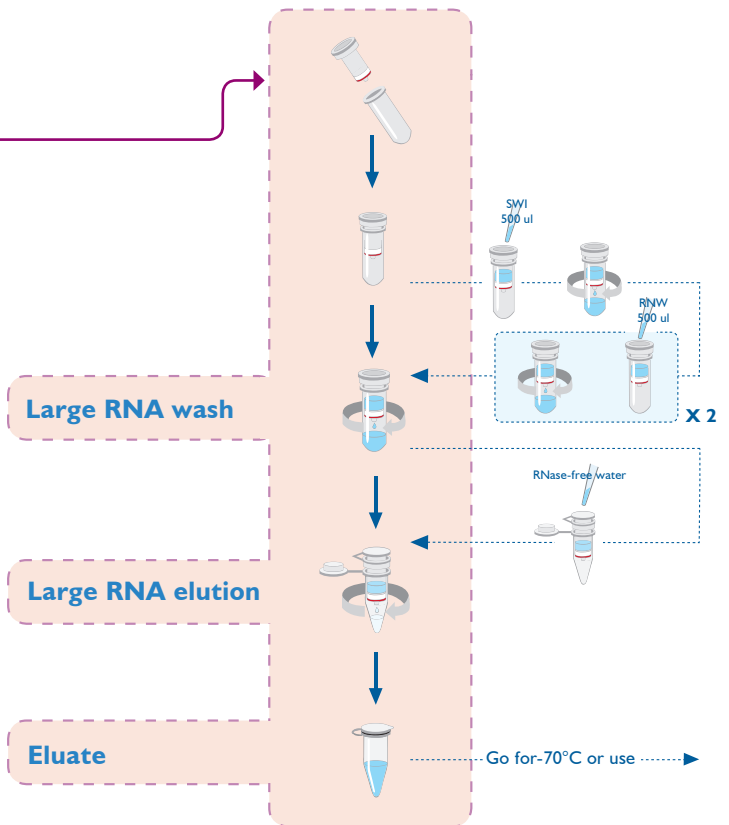
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# Brief protocol





### Large RNA Preparation



# Kit Contents

Components	Quantity	Storage
RiboEx™	30 ml	4°C
Buffer SW1	30 ml	Room temperature
Buffer RBW	30 ml	
Buffer RNW	110 ml	
RNase-free water	15 ml	
GeneAll® Column type B (red ring) (with collection tube)	50	
GeneAll® Column type W (blue ring) (with collection tube)	50	
2 ml collection tube	50	
1.5 ml collection tube	100	

## Materials Not Provided

### Reagent

- 100 % ethanol, ACS grade or better
- Equipment for homogenizing solid tissue
- Chloroform or 1-bromo-3-chloropropane (BCP)

### Disposable material

- RNase-free pipet tips
- Disposable gloves

### Equipment

- Equipment for homogenizing solid tissue
- Microcentrifuge for centrifugation at 4°C and at room temperature
- Suitable protector (ex; lab coat, disposable gloves, goggles, etc)

## Quality Control

Hybrid-R™ miRNA is manufactured in strictly clean condition, and its degree of cleanness is monitored periodically. For consistency of product, the quality certification process is carried out from lot to lot thoroughly and only the qualified is approved to be delivered.

## Storage Conditions

Hybrid-R™ miRNA kit except RiboEx™ solution should be stored at room temperature. RiboEx™ solution should be stored at 4°C for optimal performance. All components are stable for 1 year.

## User Precautions

RiboEx™ contains phenol which is poisonous and guanidine salt which is an irritant. When working with Hybrid-R™ miRNA, use gloves and eye protector to avoid contact with skin or clothing and inhalation of vapor. In case of contact, wash immediately with plenty of water and seek medical advice.

## Product Specifications

Specification	Hybird-R™ miRNA
Type	Spin
Maximum amount of starting samples	~ 100 mg or ~ $1 \times 10^7$ cells
Maximum loading volume	~ 700 $\mu$ l
Minimum elution volume	~ 30 $\mu$ l
Maximum binding capacity	~ 100 $\mu$ g

## Preventing RNase Contamination

RNase can be introduced accidentally into a RNA preparation. Wear disposable gloves always, because skin often contains bacteria that can be a source of RNase. Use sterile, disposable plasticwares and automatic pipettes reserved for RNA work to prevent cross-contamination with RNase on shared equipment.



## Product Description

In recent years, interest in small RNA, such as siRNA and miRNA which are related to research of gene regulation, has expanded. There are many commercial kits for total RNA preparation, but most of these are focused on preparation of large RNA longer than 200 nt. Because both siRNA and miRNA are between 15 ~ 30 nucleotides in length, the need of specially optimized kit for small RNA (<200 nt) is growing rapidly.

Hybrid-R™ miRNA is designed for purification of large and small RNA separately from culture cells or animal tissues, and co-purification in a single tube is also available by modified protocol. This kit utilizes the lysis method of RiboEx™ which has a powerful ability of lysis and the purification method based on glassfiber membrane technology.

Samples are homogenized in RiboEx™, a monophasic solution containing phenol and guanidium salt, which rapidly lyse cells and inactivates nucleases. Addition of chloroform brings about a separation of the lysate into aqueous and organic phases. Total RNA locates in the aqueous phase while DNA and protein remain in the interphase and organic phase. Large and small RNA in the aqueous phase is selectively bound to column type B and type W respectively. The column type B selectively adsorbs the RNA larger than 200 nt in length, while the column type W specifically holds the RNA smaller than 200 nt in length.

To purify large RNA, the aqueous phase is mixed with ethanol and the mixture is applied to a column type B. After centrifugation, large RNA is bound to membrane and the mixture containing small RNA goes into collection tube through the membrane. The membrane is washed away by two wash buffer (SW1 and RNW) and purified large RNA is eluted from the membrane by RNase-free water.

To purify small RNA, the pass-through come from the binding of large RNA is mixed with ethanol and then applied to a column type W. After washing with buffer RBW and RNW, small RNA is eluted by RNase-free water.

The procedure of Hybrid-R™ miRNA takes only 30 minutes for complete preparations of pure RNA. The purified RNA is suitable for the isolation of Poly A<sup>+</sup> RNA, Northern blotting, dot blotting, in vitro translation, cloning, RT-PCR, RPA and other analytical procedures.

# Hybrid-R™ miRNA

## PROTOCOL

for large RNA and small RNA isolation

### I. Homogenize ~ 50 mg tissue samples in 500 ul RiboEx™. Homogenize ~ $1 \times 10^7$ cells in 500 ul RiboEx™.

#### ***Tissue samples***

Basically, do not use more than 50 mg tissue per 0.5 ml RiboEx™ solution. Exceptionally for adipose tissue, up to 100 mg can be used.

- Handling fresh tissue

Immediately after dissection, inactivate RNases by any one of the following treatments.

\* Homogenize in RiboEx™ immediately.

\* Freeze rapidly in liquid nitrogen.

\* Submerge in a tissue storage buffer to protect RNA from RNases.

#### ***Cell samples***

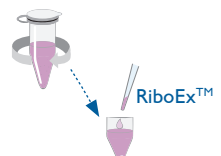
##### Cells grown in Monolayer

Pour off media, add 500 ul of RiboEx™ per 10 cm<sup>2</sup> of culture dish area. Pass the cell lysate several times through a pipette. An insufficient amount of RiboEx™ may result in contamination of the isolated RNA with DNA.

##### Cells grown in suspension

Pellet cells by centrifugation, then lyse in 500 ul of RiboEx™ per ~  $1 \times 10^7$  cultured cells by repetitive pipetting or vortexing.

\* Do not wash cells before lysing with RiboEx™ as this may contribute to mRNA degradation.



**2. Incubate the homogenate for 5 minutes at room temperature.**

This step allows nucleoprotein complexes to completely dissociate.

Homogenized samples can be stored at  $-70^{\circ}\text{C}$  for at least one month.

**3. (Optional :) Centrifuge at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to a fresh tube.**

This optional step is required only for homogenate with high contents of proteins, fats, polysaccharides or extracellular materials, such as muscles, fat, tissue, and tuberous parts of plants.

The resulting pellet contains extracellular membranes, polysaccharides, and high molecular weight DNA, while the supernatant contains RNA.

Fat tissue samples will form a layer on top of the aqueous phase. It should be removed and discarded.

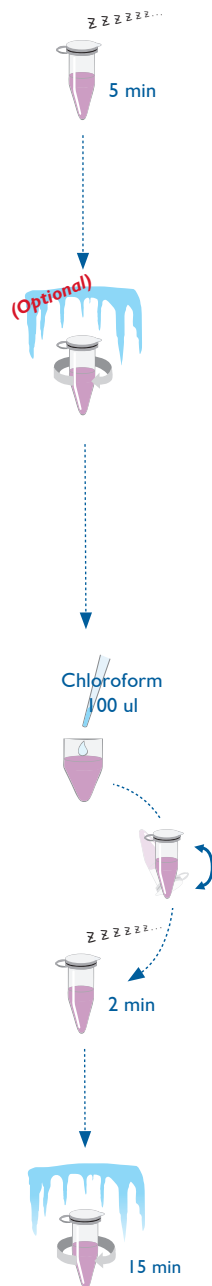
**4. Add 100  $\mu\text{l}$  of chloroform per 500  $\mu\text{l}$  of RiboEx™. Shake vigorously for 15 seconds and store for 2 minutes at room temperature.**

Alternatively, 0.05 ml of BCP (1-bromo-3-chloropropane) can be used in place of chloroform.

**5. Centrifuge at 12,000 x g for 15 minutes at 4°C and transfer the aqueous phase to a fresh tube.**

The mixture will be separated into three phases; a lower layer, an interphase, and a colorless upper aqueous layer. The upper aqueous layer is about 50% of the volume of RiboEx™ used for homogenization.

Centrifugation at over  $8^{\circ}\text{C}$  may cause some DNA to intrude in the aqueous phase.



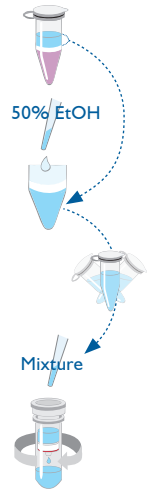
**6. Add 1 volume of 50% ethanol to the sample and mix thoroughly by inverting. Do not centrifuge.**

**7. Transfer upto 700 ul of the mixture to a mini spin column (type B, red ring).**

**8. Centrifuge at  $\geq 10,000 \times g$  for 30 seconds at room temperature. Transfer the column to a new 2 ml collection tube (provided), and store at room temperature. Use the passed-through for small (micro) RNA purification.**

Make sure that no mixture remains in the column after centrifugation. If the residual mixture has remained, centrifuge again at higher speed until all of the solution has passed through.

After this step, large RNA bind to mini spin column (type B, red ring) and small (micro) RNA exist in the passed-through.



**Go on to step 9 for small RNA purification.**

**Go on to step 21 for large RNA purification.**

### Small (micro) RNA purification (Blue ring column)



**9. Add 1 volume of 100% ethanol to the collection tube including passed-through, and mix well by pipetting. Do not centrifuge.**

**10. Transfer 650 ul of the mixture including any precipitate to a mini spin column (type W, blue ring).**

**11. Centrifuge at  $\geq 10,000 \times g$  for 30 seconds at room temperature.**

Discard the pass-through and reinsert the mini spin column back into the same tube.



**12. Repeat step 10 ~ 11 using the remainder of the sample.**

**13. Add 500  $\mu$ l of buffer RBW to the mini spin column.**

**14. Centrifuge at  $\geq 10,000 \times g$  for 30 seconds at room temperature.**

Discard the pass-through and reinsert the mini spin column back into the same tube.

**15. Add 500  $\mu$ l of buffer RNW to the mini spin column.**

**16. Centrifuge at  $\geq 10,000 \times g$  for 30 seconds at room temperature.**

Discard the pass-through and reinsert the mini spin column back into the same tube.

**17. Repeat step 15 ~ 16 once more.**

**18. Centrifuge at  $\geq 10,000 \times g$  for an additional 1 minute at room temperature to remove residual wash buffer. Transfer the mini spin column to a new 1.5 ml collection tube (provided).**

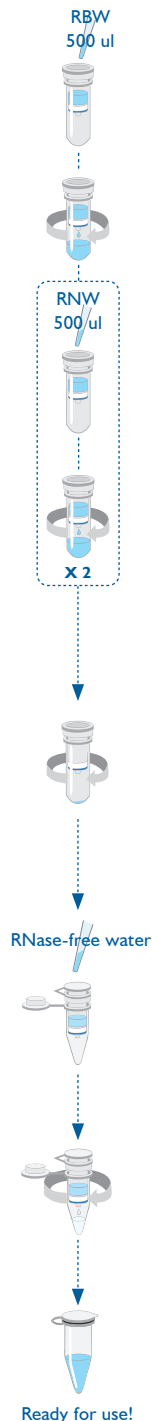
Residual ethanol may interfere with downstream reactions. Care must be taken at this step for eliminating the carryover of buffer RNW.

**19. Add 50  $\mu$ l of RNase-free water to the center of the membrane in the mini spin column.**

According to the expected yield, elution volume can be adjusted.

**20. Centrifuge at  $\geq 10,000 \times g$  for 1 minute at room temperature.**

Purified small RNA can be stored at 4°C for immediate analysis and can be stored at -70°C for long term storage.



## Large RNA purification (Red ring column)



**21. Add 500  $\mu$ l of buffer SWI to the mini spin column (type B, red ring).**

**22. Centrifuge at  $\geq 10,000 \times g$  for 30 seconds at room temperature.**

Discard the pass-through and reinsert the mini spin column back into the same tube.

**23. Add 500  $\mu$ l of buffer RNW to the mini spin column.**

**24. Centrifuge at  $\geq 10,000 \times g$  for 30 seconds at room temperature.**

Discard the pass-through and reinsert the mini spin column back into the same tube.

**25. Repeat step 23 ~ 24 once more.**

**26. Centrifuge at  $\geq 10,000 \times g$  for an additional 1 minute at room temperature to remove residual wash buffer. Transfer the mini spin column to a new 1.5 ml collection tube (provided).**

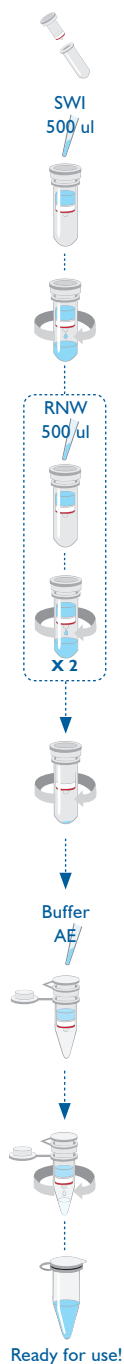
Residual ethanol may interfere with downstream reactions. Care must be taken at this step for eliminating the carryover of buffer RNW.

**27. Add 50  $\mu$ l of RNase-free water to the center of the membrane in the mini spin column.**

According to the expected yield, elution volume can be adjusted.

**28. Centrifuge at  $\geq 10,000 \times g$  for 1 minute at room temperature.**

Purified large RNA can be stored at 4°C for immediate analysis and can be stored at -70°C for long term storage.



## Troubleshooting Guide

Facts	Possible Causes	Suggestions
<b>Low yield of RNA</b>	<b>Poor quality of starting material</b>	Process the sample immediately after harvest from animal. Thaw the frozen sample directly in RiboEx™.
	<b>Sample not homogenized completely</b>	Make sure no particulate matter remains. Be sure to incubate for 5 minutes at room temperature after homogenization.
	<b>Some aqueous phase left</b>	Perform second extraction with the remaining aqueous phase.
	<b>Incorrect elution conditions</b>	Add RNase-free water to the center of the mini spin column membrane.
<b>Degradation of RNA</b>	<b>Sample manipulated too much before the addition of RiboEx™</b>	Process the sample immediately after harvest from animal.  For cultured cell, minimize washing steps. Add RiboEx™ directly to plates. Do not trypsinize cells.
	<b>Improper storage of RNA</b>	Store isolated RNA at -70°C, Do not store at -20°C.
	<b>Reagent or disposable is not RNase-free</b>	Make sure to use RNase free products only.
<b>Low A<sub>260/280</sub> (&lt;1.6)</b>	<b>Aqueous phase was contaminated with the phenol phase</b>	Avoid carryover when transferring the aqueous phase to a fresh tube.
	<b>Sample not completely homogenized with RiboEx™</b>	Use 0.5 ml RiboEx™ for up to 50 mg tissue or up to 10 <sup>6</sup> cells.
<b>Contamination of DNA</b>	<b>The interphase was co-transferred by mistake</b>	Be sure not to transfer any of the interphase (containing DNA) to the aqueous phase.

## Troubleshooting Guide

Facts	Possible Causes	Suggestions
<b>Contamination of DNA</b>	<b>Insufficient RiboEx™ used</b>	Use 0.5 ml RiboEx™ for 50 mg tissue or 10 <sup>7</sup> cells.
	<b>Temperature was too high during centrifugation</b>	The phase separation should be performed at 4°C to allow optimal separating and removal of genomic DNA from the aqueous phase.
<b>Cells not detached completely from flask after addition of RiboEx™</b>	<b>This can be seen with some strongly adherent cells</b>	After addition of RiboEx™, let cells sit 2 to 3 minutes. Scrape cells with a scraper. Incubate for several minutes. Collect and repeatedly pipette cells over flask surface. Then transfer homogenate to a tube.
<b>The yield of miRNA is too low or miRNA do not separate completely</b>	<b>Incorrect binding step</b>	Be sure to use the proper concentrations of ethanol at binding step. 50% ethanol should be used for the large RNA preparation step then 100% ethanol should be taken for the small RNA.
	<b>Too much starting sample</b>	Use 0.5 ml RiboEx™ for 50 mg tissue or 10 <sup>7</sup> cells.
<b>RNA does not perform well in downstream application</b>	<b>Residual ethanol remains in eluate</b>	Centrifuge again to remove any residual ethanol included in buffer RNW from mini spin column membrane (step 14).



# APPENDIX I • Co-purification of total RNA (Large and Small RNA)

This modified protocol allows co-purification of large and small RNA.

For the purification of total RNA, separated aqueous phase is mixed with ethanol and then the mixture is applied to column type W. Through this simple steps, total RNA is bound to the membrane. After washing steps, total RNA can be eluted by nuclease-free water.

## ■ Protocol for simultaneous purification of large RNA and small RNA from cell samples.

### I. Homogenize ~ 50 mg tissue samples in 500 ul RiboEx™.

#### Homogenize ~ $1 \times 10^7$ cells in 500 ul RiboEx™.

##### *Tissue samples*

Basically, do not use more than 50 mg tissue per 0.5 ml RiboEx™ solution.

But exceptionally for adipose tissue up to 100 mg can be used.

##### Handling fresh tissue

Immediately after dissection, inactivate RNases by any one of the following treatments.

- \* Homogenize in RiboEx™ immediately.
- \* Freeze rapidly in liquid nitrogen.
- \* Submerge in a tissue storage buffer to protect RNA from RNases.

##### *Cell samples*

##### Cells grown in Monolayer

Pour off media, add 500 ul of RiboEx™ per 10 cm<sup>2</sup> of culture dish area. Pass the cell lysate several times through a pipette. An insufficient amount of RiboEx™ may result in contamination of the isolated RNA with DNA.

##### Cells grown in suspension

Pellet cells by centrifugation, then lyse in 500 ul of RiboEx™ per ~  $1 \times 10^7$  cultured cells by repetitive pipetting or vortexing.

- \* Do not wash cells before lysing with RiboEx™ as this may contribute to mRNA degradation.

**2. Incubate the homogenate for 5 minutes at room temperature.**

This step allows nucleoprotein complexes to completely dissociate.

Homogenized samples can be stored at  $-70^{\circ}\text{C}$  for at least one month.

**3. (Optional :) Centrifuge at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to a fresh tube.**

This optional step is required only for homogenate with high contents of proteins, fats, polysaccharides or extracellular materials such as muscles, fat, tissue, and tuberous parts of plants.

The resulting pellet contains extracellular membranes, polysaccharides, and high molecular weight DNA, while the supernatant contains RNA.

Fat tissue samples will form a layer on top of the aqueous phase.

It should be removed and discarded.

**4. Add 100 ul of chloroform per 500 ul of RiboEx™. Shake vigorously for 15 seconds and store for 2 minutes at room temperature.**

Alternatively, 0.05 ml of BCP (1-bromo-3-chloropropane) can be used in place of chloroform.

**5. Centrifuge at 12,000 x g for 15 minutes at 4°C and transfer the aqueous phase to a fresh tube.**

The mixture will be separated into three phases; a lower layer, an interphase, and a colorless upper aqueous layer. The upper aqueous layer is about 50% of the volume of RiboEx™ used for homogenization.

Centrifugation at over  $8^{\circ}\text{C}$  may cause some DNA to intrude in the aqueous phase.

**6. Add 1.5 volume of 100% ethanol to the aqueous phase and mix thoroughly by inverting. Do not centrifuge.**

**7. Transfer the mixture including any precipitate to a mini spin column (type W, blue ring).**

**8. Centrifuge at  $\geq 10,000 \times g$  for 30 seconds at room temperature.**

Discard the pass-through and reinsert the mini spin column back into the same tube.

**9. Repeat step 7 ~ 8 using the remainder of the sample.**

**10. Add 500 ul of buffer RBW to the mini spin column.**

**11. Centrifuge at  $\geq 10,000 \times g$  for 30 seconds at room temperature.**

Discard the pass-through and reinsert the mini spin column back into the same tube.

**12. Add 500 ul of buffer RNW to the mini spin column.**

**13. Centrifuge at  $\geq 10,000 \times g$  for 30 seconds at room temperature.**

Discard the pass-through and reinsert the mini spin column back into the same tube.

**14. Repeat step 12 ~ 13 once more.**

**15. Centrifuge at  $\geq 10,000 \times g$  for an additional 1 minute at room temperature to remove residual wash buffer. Transfer the mini spin column to a new 1.5 ml collection tube (provided).**

Residual ethanol may interfere with downstream reactions. Care must be taken at this step for eliminating the carryover of buffer RNW.

**16. Add 50 ul of RNase-free water to the center of the membrane in the mini spin column.**

According to the expected yield, an appropriate elution volume can be applied on the membrane.

**17. Centrifuge at  $\geq 10,000 \times g$  for 1 minute at room temperature.**

Purified total RNA can be stored at 4°C for immediate analysis and can be stored at -70°C for long term storage.

## APPENDIX 2. Confirmation of RNA yield and purity by UV absorbance

### Concentration of RNA

The concentration of RNA can be determined by the absorbance at 260 nm using spectrophotometer. For the convenient measurement, we recommend using the NanoDrop® which can reduce your RNA sample and time. If unavailable, you need to dilute the RNA samples to measure the concentration through traditional spectrophotometer. The value of  $A_{260}$  should be between 0.15 and 1.00. Be sure to calibrate the spectrophotometer with the same solution used for dilution.

An absorbance of 1 at 260 nm is correspond to about 40 ug RNA / ml at a neutral pH. Therefore, the concentration of RNA was calculated by the formula shown below.

$$A_{260} \times \text{dilution factor} \times 40 = \text{RNA ug / ml}$$

### Purity of RNA

To confirm the RNA purity, you should read the ratio of  $A_{260}/A_{280}$ . Pure RNA is in the range of 1.8 ~ 2.2.

## APPENDIX 3. Formaldehyde agarose gel electrophoresis (Denaturing gel method)

A denaturing agarose gel is routinely used for the assessment of the quality of an RNA preparation. After preparation, RNA forms secondary structure via intramolecular base pairing. Therefore, it is very difficult to get the exact result of electrophoresis because of migrating inaccuracy. However, the denaturing gel denatures the secondary structure of RNA and makes an accurate migration.

To confirm the RNA band, the gel should be transferred to a UV transilluminator after electrophoresis. Mainly, two RNA bands are shown. In case of animal sample, the 28S and 18S rRNA bands are confirmed on the gel. If they are intact, the RNA bands should be sharp and the intensity of upper band should be about twice that of the lower band.

### Prepare the denaturing gel

1. Put 1g agarose in 72 ml water and heat to dissolve thoroughly.
2. Cool to 60°C.
3. Add 10 ml of 10 X MOPS buffer, 18 ml of 37% formaldehyde, and 1 ul of a 10 mg/ml ethidium bromide (EtBr).
4. Mix well then pour the gel into the gel tray and cool to solidify it.
5. Transfer the solidified gel from tray to tank, and add enough 1 X MOPS running buffer to cover the gel.

### Prepare the RNA sample

1. Make the mixture.
  - ? ul RNA (up to 20 ug)
  - 2 ul 10 X MOPS electrophoresis buffer
  - 4 ul formaldehyde
  - 10 ul formamide
2. Incubate the mixture for 15 minutes at 65°C.
3. Chill the sample for 5 minutes in ice.
4. Add 2 ul of 10 X formaldehyde gel-loading dye to the mixture.
5. Load the mixture in a denaturing gel which is covered with a sufficient 1 X MOPS electrophoresis buffer.
6. Run the gel and confirm the RNA band on transilluminator.

Occasionally, gel destaining may be needed to increase the visibility of the bands of RNA in dH<sub>2</sub>O for several hours.

## Composition of buffers

### - 10 X MOPS buffer

0.2 M MOPS

20 mM sodium acetate

10 mM EDTA

pH to 7.0 with NaOH

### - 10 X formaldehyde gel-loading dye

50% glycerol

10 mM EDTA

0.25% (w/v) bromophenol blue

0.25% (w/v) xylene cyanol FF

### \* **Caution**

When working with these chemicals, always use gloves and eye protector to avoid contact with skin and cloth. Especially, formaldehyde and ethidium bromide (EtBr) should be handled in a fume hood.

## Ordering Information

Products	Size	Type	Cat. No.
<b>GeneAll® Hybrid-Q™</b> for rapid preparation of plasmid DNA			
Plasmid Rapidprep	50	mini / spin	100-150
	200		100-102
<b>GeneAll® Exprep™</b> for preparation of plasmid DNA			
Plasmid SV mini	50	spin / vacuum	101-150
	200		101-102
	1,000		101-111
Plasmid SV Midi**	26	spin / vacuum	101-226
	50		101-250
	100		101-201
Plasmid SV Quick	50	mini / spin	101-050
	200		101-002
	1,000		101-011
<b>GeneAll® Exfection™</b> for preparation of highly pure plasmid DNA			
Plasmid LE mini (Low Endotoxin)	50	spin / vacuum	111-150
	200		111-102
Plasmid LE Midi* (Low Endotoxin)	26	spin / vacuum	111-226
	100		111-201
Plasmid EF Midi* (Endotoxin Free)	20	spin	121-220
	100		121-201
<b>GeneAll® Expin™</b> for purification of fragment DNA			
Gel SV	50	mini / spin / vacuum	102-150
	200		102-102
PCR SV	50	mini / spin / vacuum	103-150
	200		103-102
CleanUp SV	50	mini / spin / vacuum	113-150
	200		113-102
Combo GP	50	mini / spin / vacuum	112-150
	200		112-102
<b>GeneAll® Exgene™</b> for isolation of total DNA			
Tissue SV mini (plus!)*	100	spin / vacuum	104(9)-101
	250		104(9)-152
Tissue SV Midi (plus!)**	26	spin / vacuum	104(9)-226
	100		104(9)-201
Tissue SV MAXI (plus!)**	10	spin / vacuum	104(9)-310
	26		104(9)-326
Blood SV mini	100	spin / vacuum	105-101
	250		105-152
Blood SV Midi**	26	spin / vacuum	105-226
	100		105-201
Blood SV MAXI**	10	spin / vacuum	105-310
	26		105-326

## Ordering Information

Products	Size	Type	Cat. No.
<b>GeneAll® Exgene™</b> for isolation of total DNA			
Cell SV mini	100	spin / vacuum	106-101
	250		106-152
Cell SV MAXI**	10	spin / vacuum	106-310
	26		106-326
Clinic SV mini	100	spin / vacuum	108-101
	250		108-152
Clinic SV Midi	26	spin / vacuum	108-226
	100		108-201
Clinic SV MAXI**	10	spin / vacuum	108-310
	26		108-326
Genomic DNA micro	50	spin	118-050
Plant SV mini	100	spin / vacuum	117-101
	250		117-152
Plant SV Midi**	26	spin / vacuum	117-226
	100		117-201
Plant SV MAXI**	10	spin / vacuum	117-310
	26		117-326
GMO SV mini	50	spin / vacuum	107-150
	200		107-102
<b>GeneAll® GenEx™</b> for isolation of total DNA			
GenEx™ B	100 <sup>†</sup>	mini / solution	220-101
	500 <sup>†</sup>		220-105
	100 <sup>††</sup>		220-301
GenEx™ C	100 <sup>†</sup>	mini / solution	221-101
	500 <sup>†</sup>		221-105
	100 <sup>††</sup>		221-301
GenEx™ T	100 <sup>†</sup>	mini / solution	222-101
	500 <sup>†</sup>		222-105
	100 <sup>††</sup>		222-301
<b>GeneAll® DirEx™</b> Single tube DNA extraction buffer for PCR			
DirEx™	50	solution	250-050

\* GeneAll® Tissue SV mini, Midi, and MAXI plus! kit provide the additional methods for the purification from animal whole blood.

\*\* GeneAll® SV Midi / MAXI kits require the centrifuge which has a swinging-bucket rotor and ability of 4,000 ~ 5,000 xg.

† On the basis of DNA purification from 300 ul whole blood, 2 x 10<sup>8</sup> cells or 10 mg animal tissue.

†† On the basis of DNA purification from 10 ml whole blood. 1 x 10<sup>8</sup> cells or 100 mg animal tissue.



Products	Size	Type	Cat. No.
<b>GeneAll® RiboEx™</b> for preparation of total RNA			
RiboEx™	100	solution	301-001
	200		301-002
Hybrid-R™	100	spin	305-101
Hybrid-R™ Blood RNA	50	spin	315-150
Hybrid-R™ miRNA	50	spin	325-150
RiboEx™ LS	100	solution	302-001
	200		302-002
Riboclear™	50	spin	303-150
Ribospin™	50	spin	304-150
Ribospin™ vRD	50	spin	302-150
Allspin™	50	spin	306-150
<b>GeneAll® AmpONE™</b> for PCR amplification			
Taq DNA polymerase	250 U	(2.5 U/μl)	501-025
	500 U		501-050
	1,000 U		501-100
α-Taq DNA polymerase	250 U	(2.5 U/μl)	502-025
	500 U		502-050
	1,000 U		502-100
Pfu DNA polymerase	250 U	(2.5 U/μl)	503-025
	500 U		503-050
	1,000 U		503-100
Hotstart Taq DNA polymerase	250 U	(2.5 U/μl)	531-025
	500 U		531-050
	1,000 U		531-100
Clean Taq DNA polymerase	250 U	(2.5 U/μl)	551-025
	500 U		551-050
	1,000 U		551-100
Clean α-Taq DNA polymerase	250 U	(2.5 U/μl)	552-025
	500 U		552-050
	1,000 U		552-100
Taq Master mix	2x	0.5 ml x 2 tubes	511-010
	2x	0.5 ml x 10 tubes	511-050
α-Taq Master mix	2x	0.5 ml x 2 tubes	512-010
	2x	0.5 ml x 10 tubes	512-050

## Ordering Information

Products	Size	Type	Cat. No.
<b>GeneAll® AmpONE™</b> for PCR amplification			
Taq Premix	20 $\mu\ell$ 50 $\mu\ell$	96 tubes	521-200 521-500
$\alpha$ -Taq Premix	20 $\mu\ell$ 50 $\mu\ell$	96 tubes	522-200 522-500
Taq Premix (w/o dye)	20 $\mu\ell$	96 tubes	524-200
$\alpha$ -Taq Premix (w/o dye)	20 $\mu\ell$	96 tubes	525-200
dNTP mix	500 $\mu\ell$	2.5 mM each	509-020
dNTP set (set of dATP, dCTP, dGTP and dTTP)	1 ml x 4 tubes	100 mM	509-040

\* Each dNTP is available





**[www.geneall.com](http://www.geneall.com)**

Banseok Bld., 128 Oguem-dong,  
Songpa-gu, Seoul, KOREA 138-859

E-MAIL [sales@geneall.com](mailto:sales@geneall.com)

T E L 82-2-407-0096

F A X 82-2-407-0779

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Edited by SR  
Designed by Joo Sang Mi