

# Hybrid-R™ miRNA

SMALL & LARGE RNA PURIFICATION HANDBOOK

## Customer & Technical Support

Should you have any further questions, do not hesitate to contact us.

We appreciate your comments and advice.

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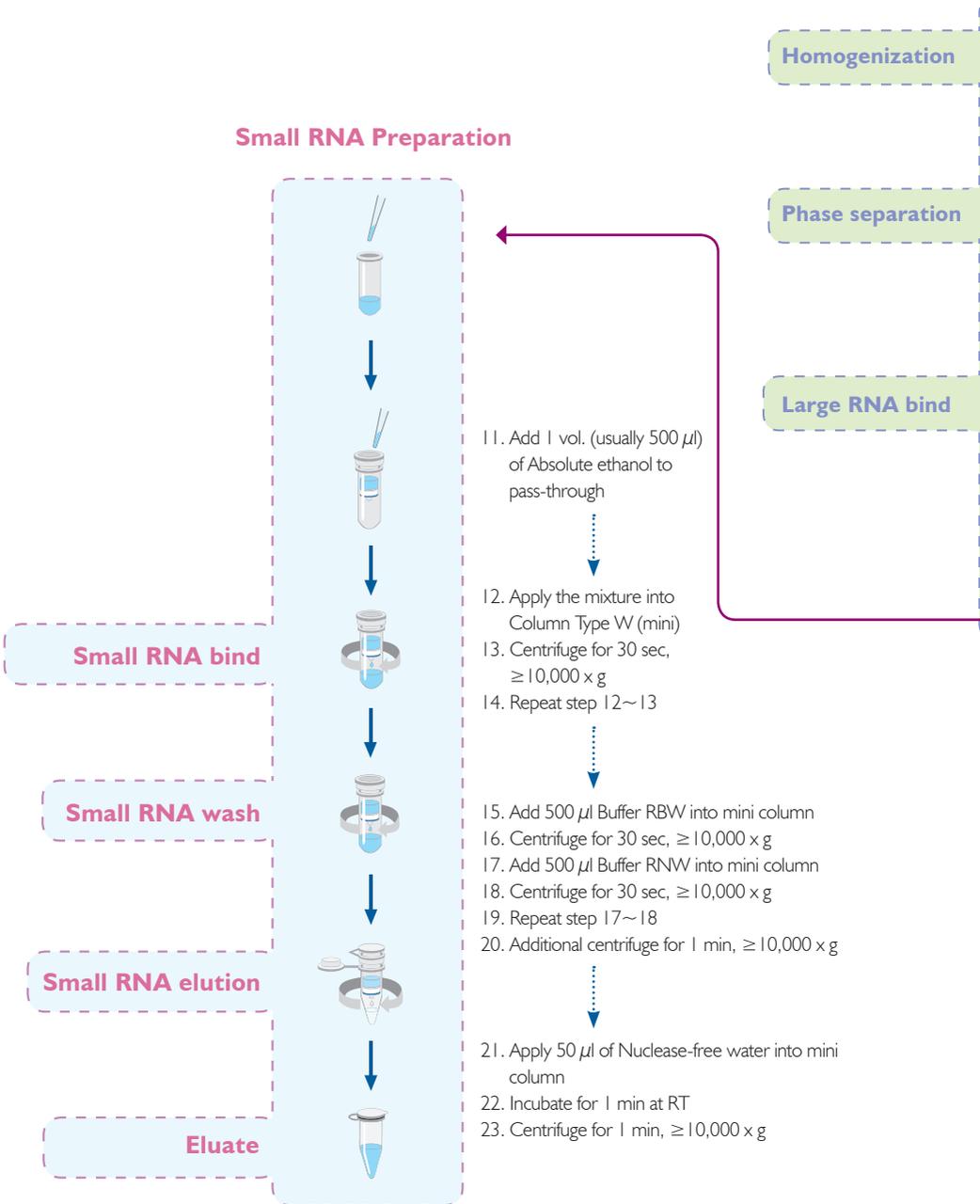
This protocol handbook is included in :

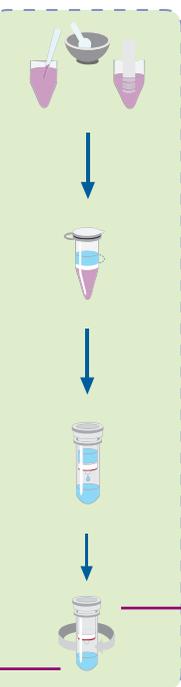
GeneAll® Hybrid-R™ miRNA (325-150)

Visit [www.geneall.com](http://www.geneall.com) for FAQ, Q&A and more information.



# Brief Protocol





1. Homogenize ~50 mg tissue samples or  $1 \times 10^7$  cells in 500  $\mu$ l RiboEx™
2. Incubate for 5 min at RT
3. (Optional) Centrifuge for 10 min at 4°C,  $\geq 12,000 \times g$

4. Add 100  $\mu$ l chloroform
5. Incubate for 2 min at RT
6. Centrifuge for 15 min at 4°C,  $\geq 12,000 \times g$
7. Transfer the aqueous phase into a new microcentrifuge tube

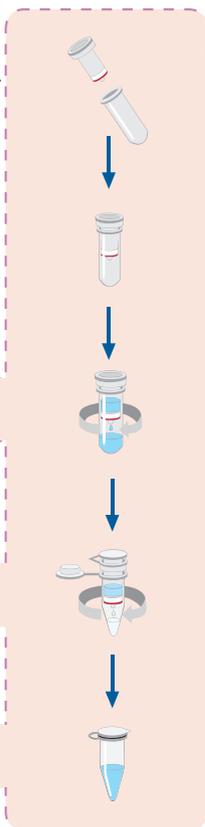
8. Add 1 vol. (usually 250  $\mu$ l) of 50% EtOH to transferred aqueous phase
9. Apply the mixture into Column Type B (mini)
10. Centrifuge for 30 sec,  $\geq 10,000 \times g$

## Large RNA Preparation

### Large RNA wash

### Large RNA elution

### Eluate



24. Add 500  $\mu$ l Buffer SW1 into mini column
25. Centrifuge for 30 sec,  $\geq 10,000 \times g$
26. Add 500  $\mu$ l Buffer RNW into mini column
27. Centrifuge for 30 sec,  $\geq 10,000 \times g$
28. Repeat step 26~27
29. Additional centrifuge for 1 min,  $\geq 10,000 \times g$

30. Apply 50  $\mu$ l Nuclease-free water into mini column
31. Incubate for 1 min at RT
32. Centrifuge for 1 min,  $\geq 10,000 \times g$



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# Kit Contents

Cat. No.	325-150	Storage
Type	mini	
Components	Quantity	
No. of preparation	50	
RiboEx™	30 ml	2~8°C
Buffer SW I	30 ml	Room temperature (15~25°C)
Buffer RBW (concentrate) *	13 ml	
Buffer RNW (concentrate) * †	22 ml	
Nuclease-free water	15 ml	
Column Type B (mini) (with collection tube)	50	
Column Type W (mini) (with collection tube)	50	
2 ml collection tube	50	
1.5 ml microcentrifuge tube	100	
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\* Before first use, add absolute ethanol (ACS grade or better) into Buffer RBW and RNW as indicated on the bottle.

† Contains sodium azide as a preservative.

## Materials Not Provided

### Reagents

- Absolute ethanol (ACS grade or better)
- Chloroform or 1-bromo-3-chloropropane (BCP)

### Disposable materials

- RNase-free pipette tips
- Disposable gloves
- Sterile 1.5 ml microcentrifuge tubes

### Equipments

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

## Product Specifications

Hybrid-R™ miRNA		
Specification	Column Type B (mini) for Large RNA	Column Type W (mini) for Small RNA
Type	Spin	Spin
Maximum amount of starting samples	Solid sample : 100 mg/prep Cultured cell : $1 \times 10^7$ /prep	Solid sample : 100 mg/prep Cultured cell : $1 \times 10^7$ /prep
Preparation time	$\geq 30$ min	$\geq 30$ min
Maximum loading volume of mini column	700 $\mu$ l	700 $\mu$ l
Minimum elution volume of mini column	50 $\mu$ l	30 $\mu$ l
Maximum binding capacity of mini column	100 $\mu$ g	100 $\mu$ g

## Quality Control

All components in Hybrid-R™ miRNA are manufactured in strictly clean conditions, and its degree of cleanness is monitored periodically. Quality control is carried out thoroughly from lot to lot, and only the qualified kits are approved to be delivered.

## Storage Conditions

All components of Hybrid-R™ miRNA (except RiboEx™ solution) should be stored at room temperature (15~25°C). It should be protected from exposure to direct sunlight.

RiboEx™ solution should be stored at 2~8°C for optimal performance.

During shipment or storage under cool ambient condition, a precipitate can be formed in Buffer RBW. In such a case, heat the bottle to 50°C to dissolve completely. Using precipitated buffers will lead to poor DNA recovery.

Hybrid-R™ miRNA is guaranteed until the expiration date printed on the product box.

## Safety Information

The buffers included in Hybrid-R™ miRNA contain irritants which are harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken when handling such materials. Always wear gloves and eye protection, and follow standard safety precautions.

RiboEx™ contains phenol which is poisonous and RiboEx™, Buffer RBW, and SWI contain chaotropic agents, which can form highly reactive compounds when combined with bleach.

Do NOT add bleach or acidic solutions directly to the sample-preparation waste.

## Product Disclaimer

Hybrid-R™ miRNA is for research use only, not for use in diagnostic procedure.

## Prevention of RNase Contamination

RNase can be introduced accidentally during RNA purification. Wear disposable gloves always, because skin often contains bacteria and molds that can be a source of RNase contamination. Use sterile, disposable plastic wares and automatic pipettes to prevent cross-contamination of RNase from shared equipment.

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 (nucleotides). Because both siRNA and miRNA are between 15~30 nt 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 cultured 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 glass fiber 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 buffers (Buffer SW1 and Buffer RNW) and purified large RNA is eluted from the membrane by Nuclease-free water.

To purify small RNA, the pass-through come from the binding step 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 Nuclease-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 $\mu$ l RiboEx™. Homogenize ~1 x 10<sup>7</sup> cells in 500 $\mu$ l 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**

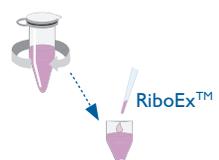
###### Cells grown in Monolayer

Pour off media, add 500  $\mu$ l 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  $\mu$ l of RiboEx™ per ~1 x 10<sup>7</sup> cultured cells by repetitive pipetting or vortexing.

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



**2. Incubate the homogenate for 5 min 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 min at  $4^{\circ}\text{C}$  and transfer the supernatant to a fresh 1.5 ml microcentrifuge tube (not provided).**

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.

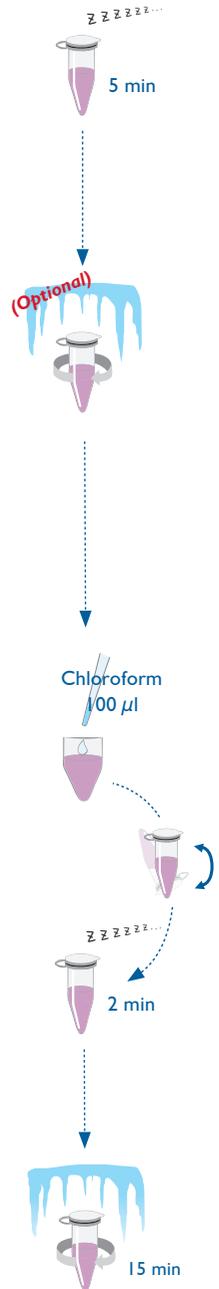
**4. Add 100  $\mu\text{l}$  of chloroform per 500  $\mu\text{l}$  of RiboEx™. Shake vigorously for 15 sec and incubate for 2 min at room temperature.**

Alternatively, 50  $\mu\text{l}$  of BCP (1-bromo-3-chloropropane) can be used in place of chloroform.

**5. Centrifuge at 12,000 x g for 15 min at  $4^{\circ}\text{C}$  and transfer the aqueous phase to a fresh 1.5 ml microcentrifuge tube (not provided).**

The mixture will be separated into three phases; a lower phase, an interphase, and a colorless upper aqueous phase. 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.



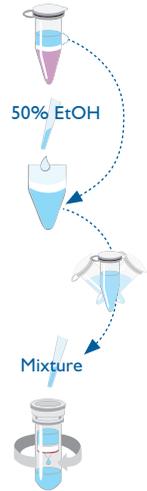
6. Add 1 volume (usually 250  $\mu$ l) of 50% ethanol to the transferred aqueous phase and mix thoroughly by inverting. Do NOT centrifuge.

7. Transfer all the mixture to a Column Type B (mini).

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

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

After this step, large RNA bind to mini column and small (micro) RNA exist in the pass-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 (usually 500  $\mu$ l) of absolute ethanol to the collection tube including pass-through, and mix well by pipetting. Do NOT centrifuge.

10. Transfer 650  $\mu$ l of the mixture including any precipitate to a Column Type W (mini).

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

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



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

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

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

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

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

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

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

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

**18. Centrifuge at  $\geq 10,000 \times g$  for an additional 1 min at room temperature to remove residual wash buffer. Transfer the mini column to a new 1.5 ml microcentrifuge 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 Nuclease-free water to the center of the membrane in the mini column. Incubate for 1 min at room temperature.**

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

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

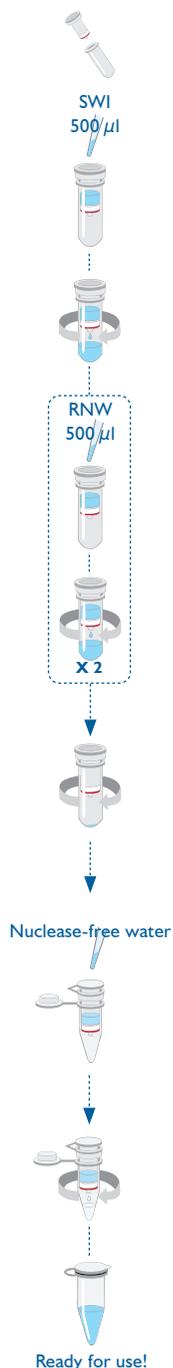
Purified small RNA can be stored at 4°C for immediate analysis and can be stored at -70°C for long term storage.



## Large RNA purification (Red ring column)



- 21. Add 500  $\mu$ l of Buffer SW1 to the Column Type B (mini).**
- 22. Centrifuge at  $\geq 10,000 \times g$  for 30 sec at room temperature.**  
Discard the pass-through and reinsert the mini column back into the collection tube.
- 23. Add 500  $\mu$ l of Buffer RNW to the mini column.**
- 24. Centrifuge at  $\geq 10,000 \times g$  for 30 sec at room temperature.**  
Discard the pass-through and reinsert the mini column back into the collection tube.
- 25. Repeat step 23~24.**
- 26. Centrifuge at  $\geq 10,000 \times g$  for an additional 1 min at room temperature to remove residual wash buffer. Transfer the mini column to a new 1.5 ml microcentrifuge 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 Nuclease-free water to the center of the membrane in the mini column. Incubate for 1 min at room temperature.**  
According to the expected yield, an appropriate elution volume can be applied on the membrane.
- 28. Centrifuge at  $\geq 10,000 \times g$  for 1 min 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>Insufficient homogenizing of sample</b>	Make sure no particulate matter remains. Be sure to incubate for 5 min 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 Nuclease-free water to the center of the mini 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 products 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>Insufficient lysis of sample with RiboEx™</b>	Use 0.5 ml RiboEx™ for up to 50 mg tissue or up to 1 × 10 <sup>7</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.

Facts	Possible Causes	Suggestions
<b>Contamination of DNA</b>	<b>Insufficient RiboEx™ used</b>	Use 0.5 ml RiboEx™ for 50 mg tissue or $1 \times 10^7$ 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 min. 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 absolute ethanol should be used for the small RNA.
	<b>Too much starting sample</b>	Use 0.5 ml RiboEx™ for 50 mg tissue or $1 \times 10^7$ 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 column membrane (step 18, 26).

# 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 of tissue samples in 500 $\mu$ l RiboEx™. Homogenize ~1 x 10<sup>7</sup> cells in 500 $\mu$ l 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  $\mu$ l 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  $\mu$ l of RiboEx™ per ~1 x 10<sup>7</sup> cultured cells by repetitive pipetting or vortexing.

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

**2. Incubate the homogenate for 5 min 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 min at 4°C and transfer the supernatant to a fresh 1.5 ml microcentrifuge tube (not provided).**

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 sec and incubate for 2 min at room temperature.**

Alternatively, 50  $\mu\text{l}$  of BCP (1-bromo-3-chloropropane) can be used in place of chloroform.

**5. Centrifuge at 12,000 x g for 15 min at 4°C and transfer the aqueous phase to a fresh 1.5 ml microcentrifuge tube (not provided).**

The mixture will be separated into three phases; a lower phase, an interphase, and a colorless upper aqueous phase. The upper aqueous phase 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 (usually 375  $\mu\text{l}$ ) of absolute ethanol to the transferred aqueous phase and mix thoroughly by inverting. Do NOT centrifuge.**

**7. Transfer all the mixture including any precipitate to a Column Type W (mini).**

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

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

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

**10. Add 500  $\mu$ l of Buffer RBW to the mini column.**

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

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

**12. Add 500  $\mu$ l of Buffer RNW to the mini column.**

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

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

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

**15. Centrifuge at  $\geq 10,000 \times g$  for an additional 1 min at room temperature to remove residual wash buffer. Transfer the mini column to a new 1.5 ml microcentrifuge 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  $\mu$ l of Nuclease-free water to the center of the membrane in the mini column. Incubate for 1 min at room temperature.**

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 min 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  $\mu\text{g}$  RNA/ml at a neutral pH. Therefore, the concentration of RNA was calculated by the formula shown below.

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

### 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 1 g agarose in 72 ml water and heat to dissolve thoroughly.
2. Cool to 60°C.
3. Add 10 ml of 10X MOPS buffer, 18 ml of 37% formaldehyde, and 1  $\mu$ l 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 1X MOPS running buffer to cover the gel.

### Prepare the RNA sample

1. Make the mixture.
  - ?  $\mu$ l RNA (up to 20  $\mu$ g)
  - 2  $\mu$ l 10X MOPS electrophoresis buffer
  - 4  $\mu$ l formaldehyde
  - 10  $\mu$ l formamide
2. Incubate the mixture for 15 min at 65°C.
3. Chill the sample for 5 min in ice.
4. Add 2  $\mu$ l of 10X formaldehyde gel-loading dye to the mixture.
5. Load the mixture in a denaturing gel which is covered with a sufficient 1X 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

### - 10X MOPS buffer

0.2 M MOPS

20 mM sodium acetate

10 mM EDTA

pH to 7.0 with NaOH

### - 10X 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	Scale	Size	Cat. No.	Type
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### GeneAll® Hybrid-Q™ for rapid preparation of plasmid DNA

Plasmid Rapidprep	mini	50	100-150	spin
		200	100-102	

### GeneAll® Exprep™ for preparation of plasmid DNA

Plasmid SV	mini	50	101-150	spin /	
		200	101-102	vacuum	
	Midi	26	101-226	spin /	vacuum
		50	101-250		
		100	101-201		
		MAXI	10		

### GeneAll® Exfection™

for preparation of transfection-grade plasmid DNA

Plasmid LE (Low Endotoxin)	mini	50	111-150	spin /
		200	111-102	vacuum
	Midi	26	111-226	spin /
		100	111-201	vacuum
Plasmid EF (Endotoxin Free)	Midi	20	121-220	spin
		100	121-201	

### GeneAll® Expin™ for purification of fragment DNA

Gel SV	mini	50	102-150	spin /
		200	102-102	vacuum
PCR SV	mini	50	103-150	spin /
		200	103-102	vacuum
CleanUp SV	mini	50	113-150	spin /
		200	113-102	vacuum
Combo GP	mini	50	112-150	spin /
		200	112-102	vacuum

### GeneAll® Exgene™ for isolation of total DNA

Tissue SV	mini	100	104-101	spin /
		250	104-152	vacuum
	Midi	26	104-226	spin /
		100	104-201	vacuum
	MAXI	10	104-310	spin /
		26	104-326	vacuum
Tissue Plus SV	mini	100	109-101	spin /
		250	109-152	vacuum
	Midi	26	109-226	spin /
		100	109-201	vacuum
	MAXI	10	109-310	spin /
		26	109-326	vacuum

Products	Scale	Size	Cat. No.	Type
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### GeneAll® Exgene™ for isolation of total DNA

Blood SV	mini	100	105-101	spin /	
		250	105-152	vacuum	
	Midi	26	105-226	spin /	
		100	105-201	vacuum	
	MAXI	10	105-310	spin /	
		26	105-326	vacuum	
Cell SV	mini	100	106-101	spin /	
		250	106-152	vacuum	
	MAXI	10	106-310	spin /	
		26	106-326	vacuum	
	Clinic SV	mini	100	108-101	spin /
			250	108-152	vacuum
Midi		26	108-226	spin /	
		100	108-201	vacuum	
MAXI		10	108-310	spin /	
		26	108-326	vacuum	
Genomic DNA micro	mini	50	118-050	spin	
		100	117-101	spin /	
	Midi	250	117-152	vacuum	
		26	117-226	spin /	
	MAXI	100	117-201	vacuum	
		10	117-310	spin /	
26	117-326	vacuum			
Soil DNA mini	mini	50	114-150	spin	
Stool DNA mini	mini	50	115-150	spin	
Viral DNA/RNA	mini	50	128-150	spin	
FFPE Tissue DNA	mini	50	138-150	spin	
		250	138-152		

### GeneAll® GenEx™ for isolation of total DNA without spin column

GenEx™ Blood	Sx	100	220-101	solution
		500	220-105	
GenEx™ Cell	Sx	100	221-101	solution
		500	221-105	
GenEx™ Tissue	Sx	100	222-101	solution
		500	222-105	
GenEx™ Blood	Lx	100	220-301	solution
		100	221-301	
GenEx™ Cell	Lx	100	221-301	solution
		100	222-301	

Products	Scale	Size	Cat. No.	Type
<b>GeneAll® GenEx™</b> <i>for isolation of total DNA without spin column</i>				
GenEx™ Plant	Sx	100	227-101	solution
	Mx	100	227-201	
	Lx	100	227-301	
GenEx™ Plant Plus	Sx	100	228-101	solution
	Mx	50	228-250	
	Lx	20	228-320	

**GeneAll® DirEx™ series** *for preparation of PCR-template without extraction*

DirEx™	100	250-101	solution
DirEx™ Fast-Tissue	96 T	260-011	solution
DirEx™ Fast-Cultured cell	96 T	260-021	solution
DirEx™ Fast-Whole blood	96 T	260-031	solution
DirEx™ Fast-Blood stain	96 T	260-041	solution
DirEx™ Fast-Hair	96 T	260-051	solution
DirEx™ Fast-Buccal swab	96 T	260-061	solution
DirEx™ Fast-Cigarette	96 T	260-071	solution

**GeneAll® RNA series** *for preparation of total RNA*

RiboEx™	mini	100	301-001	solution
		200	301-002	
Hybrid-R™	mini	100	305-101	spin
Hybrid-R™ Blood RNA	mini	50	315-150	spin
Hybrid-R™ miRNA	mini	50	325-150	spin
RiboEx™ LS	mini	100	302-001	solution
		200	302-002	
Riboclear™	mini	50	303-150	spin
Riboclear™ Plus	mini	50	313-150	spin
Ribospin™	mini	50	304-150	spin
Ribospin™ II	mini	50	314-150	spin
		300	314-103	
Ribospin™ vRD	mini	50	302-150	spin
Ribospin™ vRD Plus	mini	50	312-150	spin
Ribospin™ vRD II	mini	50	322-150	spin
Ribospin™ Plant	mini	50	307-150	spin
Ribospin™ Seed/Fruit	mini	50	317-150	spin
Ribospin™ Pathogen/TNA	mini	50	314-150	spin
		250	314-152	
Allspin™	mini	50	306-150	spin
RiboSaver™	mini	100	351-001	solution

Products	Scale	Size	Cat. No.	Type
<b>GeneAll® AmpONE™</b> <i>for PCR amplification</i>				
Taq DNA polymerase		250 U	501-025	(2.5 U/μl)
		500 U	501-050	
		1,000 U	501-100	
Taq Premix		20 μl × 96 tubes	526-200	solution
		50 μl × 96 tubes	526-500	

**GeneAll® AmpMaster™** *for PCR amplification*

Taq Master mix	0.5 ml × 2 tubes	541-010	solution
	0.5 ml × 10 tubes	541-050	solution

**GeneAll® HyperScript™** *for Reverse Transcription*

Reverse Transcriptase	10,000 U	601-100	solution
RT Master mix	0.5 ml × 2 tubes	601-710	solution
One-step RT-PCR Master mix	0.5 ml × 2 tubes	602-110	solution
One-step RT-PCR Premix	20 μl × 96 tubes	602-102	solution

**GeneAll® RealAmp™** *for qPCR amplification*

SYBR qPCR Master mix (2X, Low ROX)	200 rxn	2 ml	801-020	solution
	500 rxn	5 ml	801-050	
SYBR qPCR Master mix (2X, High ROX)	200 rxn	2 ml	801-021	solution
	500 rxn	5 ml	801-051	

Products	Size	Cat. No.	Type
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### GeneAll® Protein series

ProteinEx™ Animal cell/tissue	100 ml	701-001	solution
PAGESTA™ Reducing 5X SDS-PAGE Sample Buffer	1 ml × 10 tubes	751-001	solution

### GeneAll® STEADi™ for automatic nucleic acid purification

12 Instrument		GST012	system
24 Instrument		GST024	system
Genomic DNA Cell/Tissue	96	401-104	kit
Genomic DNA Blood	96	402-105	kit
Total RNA	96	404-304	kit
Viral DNA / RNA	96	405-322	kit
CFC Seed DNA/RNA	96	406-C02	kit
Genomic DNA Plant	96	407-117	kit
Soil DNA	96	408-114	kit

### GeneAll® GENTi™ 32 *Ultimately flexible automatic extraction system*

Automatic extrantion equipment		GTI032	system
Genomic DNA	48	901-048	tube
	96	901-096	plate
Viral DNA/RNA	48	902-048	tube
	96	902-096	plate
Whole Blood Genomic DNA	48	903-048	tube
	96	903-096	plate

Products	Scale	Size	Cat. No.	Type
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### GeneAll® GENTi™ 32 *Ultimately flexible automatic extraction system*

Automatic extrantion equipment			GTI032A	system
Genomic DNA	48		901-048A	tube
	96		901-096A	plate
Viral DNA/RNA	48		902-048A	tube
	96		902-096A	plate
Blood DNA	48		903-048A	tube
	96		903-096A	plate
Plant DNA/RNA	48		904-048A	tube
	96		904-096A	plate
LMO	48		906-048A	tube
	96		906-096A	plate



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