Ver 1.2

# Ribospin<sup>TM</sup> II

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® Ribospin™ II (314-150, 314-103)

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### **Animal Cell**

1. Prepare animal cell (5 x  $10^6 \sim 1 \times 10^7$  cells)



### Lyse





- 2. Add 350  $\mu$ l (700  $\mu$ l) Buffer RAL
- 3. Add I volume of 70% ethanol



Bind



4. Transfer the lysate into Column Type F (mini)

5. Centrifuge at  $\geq$  10,000 x g for 1 min





### treatment

- 6. Add 350  $\mu$ I Buffer RW
- 7. Centrifuge at  $\geq$  10,000 x g for 30 sec
- 8. Apply 70  $\mu$ l DNase I reaction mixture into mini column
- 9. Incubate for 10 min at RT





Elute







- 10. Add 350  $\mu$ l Buffer RW II. Centrifuge at  $\geq$  10,000 x g for 30 sec

  - 12. Add 500  $\mu$ l Buffer RSW
  - 13. Centrifuge at  $\geq$  10,000 x g for 30 sec
  - 14. Repeat step 12~13
  - 15. Additional centrifuge at  $> 13,000 \times g$  for 1 min



- 16. Apply 50  $\mu$ l Nuclease-free water into mini column
- 17. Incubate for 1 min at RT
- 18. Centrifuge at  $\geq$  10,000 x g for 1 min

### **Animal Tissue**

- I. Homogenize animal tissue (20~30 mg) in 350 μl (700 μl) Buffer RAL (including 1% \(\beta\)-mercaptoethanol)
- 2. Centrifuge at  $\geq 10,000 \times g$  for 2 min
- 3. Transfer the supernatant into a new microcentrifuge tube
- 4. Add 1 volume of 70% ethanol



- 5. Transfer the lysate into Column Type F (mini)
- 6. Centrifuge at  $\geq 10,000 \times g$  for 1 min



- 7. Add 350  $\mu$ l Buffer RW
- 8. Centrifuge at  $\geq 10,000 \times g$  for 30 sec
- 9. Apply 70  $\mu$ l DNase I reaction mixture into mini column
- 10. Incubate for 10 min at RT



- II. Add 350  $\mu$ I Buffer RW
- 12. Centrifuge at  $\geq$  10,000 x g for 30 sec
- 13. Add 500  $\mu$ l Buffer RSW
- 14. Centrifuge at  $\geq$  10,000 x g for 30 sec
- 15. Repeat step 13~14
- 16. Additional centrifuge at  $> 13,000 \times g$  for 1 min



- 17. Apply 50  $\mu$ l Nuclease-free water into mini column
- 18. Incubate for 1 min at RT
- 19. Centrifuge at  $\geq$  10,000 x g for 1 min



Pure RNA

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## Kit Contents

Cat. No.	314-150	314-103	
Туре	mini	mini	Storage
Components	Qua		
No. of preparation	50	300	
Column Type F (mini) (with collection tube)	50	300	
1.5 ml microcentrifuge tube	50	300	
Buffer RAL	40 ml	240 ml	5
Buffer RW	40 ml	240 ml	Room
Buffer RSW (concentrate) *	I2 ml	36 ml x 2	temperature (15~25°C)
Nuclease-free water	I5 ml	90 ml	(13°-23°C)
Buffer DRB	5 ml	30 ml	
DNase I (lyophilized) **	240 Kunitz units	1,440 Kunitz units	
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<sup>\*</sup> Before first use, add absolute ethanol (ACS grade or better) into Buffer RSW as indicated on the bottle.

#### **Materials Not Provided**

- Reagent : β-mercaptoethanol, 70% ethanol, Absolute ethanol (ACS grade or better)
- Disposable material : RNase-free pipette tips, Sterile 1.5 ml microcentrifuge tubes, Disposable gloves
- Equipment : Equipment for homogenizing sample, Microcentrifuge, Vortex mixer, Suitable protector

### **Product Specifications**

Ribospin™ II					
Туре	Spin				
Maximum amount of starting samples	$1 \times 10^7$ cells or 30 mg tissue/prep				
Preparation time	≥30 min				
Maximum loading volume of mini column	750 <i>µ</i> l				
Minimum elution volume	30 <i>µ</i> I				
Maximum binding capacity	500 μg				

<sup>\*</sup> Contains sodium azide as a preservative.

<sup>\*\*</sup> For the long-term storage of lyophilized DNase I, store at 4°C. But after reconstitution of DNase I, store at -20°C.

Refer to instruction of DNase I on page 8 and I 0.

## **Quality Control**

All components in Ribospin $^{TM}$  II 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 Ribospin<sup>TM</sup> II should be stored at room temperature ( $15\sim25^{\circ}$ C). It should be protected from exposure to direct sunlight.

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

 $\mathsf{Ribospin}^{\mathsf{TM}} \mathsf{II}$  is guaranteed until the expiration date printed on the product box.

## **Safety Information**

The buffers included in the Ribospin<sup>™</sup> II 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. Buffer RAL and RW contains chaotropes 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**

 $Ribospin^{TM} \ II \ is \ for \ research \ use \ only, \ not. for .use. in. diagnostic \ procedure....$ 

### **Preventing 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.

## **Preparation of DNase I Solution**

The DNase I is provided in a lyophilized format. It should be reconstituted thoroughly with Nuclease-free water (provided for RNA elution) before experiment.

To obtain DNase I solution, add 120  $\mu$ I (Cat. No. 314-150) Nuclease-free water to the tube containing lyophilized DNase I (240 Kuniz units), and mix carefully and gently to avoid foaming.

Dissolve the DNase I thoroughly, divide it into conveniently sized aliquots, and store at -20°C. For one preparation, 2  $\mu$ I DNase I solution is required.

## **Product Description**

Ribospin<sup>TM</sup> II is devised to purify RNA from cultured cells or animal tissues ( $\sim 1 \times 10^7$  cells or  $\sim 30$  mg Tissue). With the GeneAll's glassfiber membrane technology, highly pure RNA can be conveniently isolated in less than 30 minutes instead of the time consuming and hazardous conventional methods which require alcohol precipitation or toxic chemicals such as phenol/chloroform.

The optimized buffer system of Ribospin<sup>™</sup> II maximizes the specific binding efficiency of RNA to the glassfiber membrane but minimizes the contamination of impurities by a series of optimized wash buffer. Also, the contaminated DNA residues can be easily eliminated during the preparation by on-column digestion using DNase I included in this kit. Pure RNA which finally prepared in Nuclease-free water can be applied to the most of downstream application which require the pure RNA, and this whole procedure can be completely performed at room temperature.

The purified RNA should be treated with care because RNA is relatively unstable and fragile. It is strongly recommended to store the eluate at 4°C for immediate analysis or at -70°C for long-term storage.

We strongly recommend reading the procedure to using Ribospin  $^{\text{TM}}$  II.

# Protocol for total RNA purification with On-column DNase I treatment from animal cell

### Before experiment

- Prepare DNase I reaction mixture as below;
  - 1) Thaw a working solution of DNase I on ice
  - ② Mix 2  $\mu$ l of DNase I solution with 70  $\mu$ l of Buffer DRB per preparation
  - 3 Mix gently by pipetting without vortex.
    - v Make the mixture as just before step 7 as possible
    - v Treat DNase I always on ice

### I. Harvest cell samples in a tube.

### Cells grown in monolayer

Harvest  $5 \times 10^6$  cells carefully using scraper, pellet cells by centrifugation at low speed (below  $800 \times g$ ) for 5 minutes, and then discard the culture medium.

### Cells grown in suspension

Pellet  $5 \times 10^6$  cells by centrifugation at low speed (below  $800 \times g$ ) for 5 minutes, and then discard the culture medium.

## 2. Add 350 or 700 $\mu$ I of Buffer RAL (Refer to Table I) to the tube and lyse the sample by pipetting or micro-homogenizer.

Lyse the 5 x  $10^6$  cells in 350  $\mu$ l Buffer RAL. An insufficient lysis may result in low RNA recovery rate or mini column clogging.

Table 1. Reagent volumes for sample amounts

Cell numbers	Buffer RAL
$\sim$ 5 x 10 $^{6}$ cells	350 <i>µ</i> l
$5 \times 10^6 \sim 1 \times 10^7$ cells	700 µl

<sup>\*</sup> Do not wash the cells before lysing with Buffer RAL as this may cause mRNA degradation.

- 3. Add I volume (usually 350 or 700  $\mu$ I) of 70% ethanol to the lysate and mix well by pipetting. Do not centrifuge.
- 4. Transfer 750  $\mu$ l of the mixture to a Column Type F (mini). If the mixture volume exceeds 750  $\mu$ l, repeat the step 4~5 with the remainder of the sample.
- 5. Centrifuge at  $\geq 10,000 \times g$  for 1 min at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.
- 6. Add 350  $\mu$ l of Buffer RW and centrifuge at  $\geq$  10,000 x g for 30 sec. Discard the pass-through and reinsert the mini column back into the collection tube.
- 7. Add 70  $\mu$ l of DNase I reaction mixture on to the center of the mini column membrane and incubate for 10 min at room temperature.
  - To make DNase I reaction mixture, add 2  $\mu$ I of DNase I solution to 70  $\mu$ I of Buffer DRB per isolation. And keep it on ice to protect the activity of DNase I until use.
- 8. Add 350  $\mu$ l of Buffer RW and centrifuge at  $\geq$  10,000 x g for 30 sec. Discard the pass-through and reinsert the mini column back into the collection tube.
- 9. Add 500  $\mu$ l of Buffer RSW and centrifuge at  $\geq$  10,000 x g for 30 sec. Discard the pass-through and reinsert the mini column back into the collection tube.
- 10. Add 500  $\mu$ l of Buffer RSW again and centrifuge at  $\geq$ 10,000 x g for 30 sec. Discard the pass-through and reinsert the mini column back into the collection tube.
- 11. Centrifuge at full speed (>13,000 x g) for 1 min to remove residual wash buffer. Place the mini column into a fresh 1.5 ml microcentrifuge tube (provided).

Residual ethanol may interfere with downstream applications. Care must be taken at this step for eliminating the carryover of Buffer RSW.

### 12. Add 50 $\mu$ l of Nuclease-free water to the center of the membrane in the mini column. Incubate at the room temperature for I min.

Elution volume can be adjusted according to an experiment's purpose.

Using the eluent volume of less than 50  $\mu$ l will be decrease the total RNA yield but increase the concentration of RNA. But for effective elution of RNA, more than 30  $\mu$ l of the eluent should be applied, because too lower volume of eluent cannot soak the membrane completely.

### 13. Centrifuge at $\geq$ 10,000 x g for 1 min at room temperature.

Purified RNA can be stored at 4°C for immediate analysis, otherwise it is recommended to store at -70°C for long-term storage.

# Protocol for total RNA purification with On-column DNase I treatment from animal tissue

### Before experiment

- The protocol is suitable for fresh, frozen and stabilized tissue sample in RiboSaver<sup>™</sup>.
- In case that the preserved sample in RNA stabilization solution like RiboSaver<sup>™</sup>, the stabilization solution should be discarded completely.
- Make 1%  $\beta$ -mercaptoethanol (ex, 10  $\mu$ l per 1 ml) with Buffer RAL before every experiment.
- Prepare DNase I reaction mixture as below;
  - ① Thaw a working solution of DNase I on ice
  - 2) Mix 2  $\mu$ l of DNase I solution with 70  $\mu$ l of Buffer DRB per preparation
  - 3 Mix gently by pipetting without vortex.
    - v Make the mixture as just before step 7 as possible
    - v Treat DNase I always on ice

### 1. Homogenize ~20 mg of tissue as described in step Ia, Ib, or Ic.

Thoroughly disrupt the tissue in Buffer RAL and lyse the samples perfectly. Unclarified sample may cause clogging of the mini column in subsequent steps.

For the effective application of fiber-rich tissues (ex, heart, muscle, skin), we strongly recommend to use up to 10 mg per preparation. If using more than 10 mg, the lysate would not be clarified completely and it will lead to clogging of spin mini column membrane.

Table 2. Reagent volumes for tissue amounts

Tissue amounts	Buffer RAL (including I % β-mercaptoethanol)
~20 mg	350 µl
20 mg~30 mg	$700\mu$ l

Ia. Grind the tissue sample to a fine powder with liquid nitrogen in a pre-chilled mortar and pestle. Put up to 20 mg of the powdered tissue into 1.5 ml microcentrifuge tube. Add 350 or 700  $\mu$ l of Buffer RAL (Refer to Table 2) (including 1%  $\beta$ -mercaptoethanol) and pulse-vortex for 30 sec.

- Ib. Homogenize up to 20 mg of the tissue sample in 350 or 700  $\mu$ l of Buffer RAL (including 1% β-mercaptoethanol) using homogenizer.
- Ic. Homogenize the tissue sample in 2.0 ml collection tube using bead-beater. Add 350 or 700  $\mu$ l of Buffer RAL (including 1%  $\beta$ -mercaptoethanol) and pulse-vortex for 30 sec.
- 2. Centrifuge at  $\geq 10,000 \times g$  for 2 min at room temperature and transfer the supernatant to a fresh 1.5 ml microcentrifuge tube (not provided).

This step can help avoid clogging of a mini column caused by incompletely homogenized debris.

- 3. Add I volume (usually 350 or 700  $\mu$ I) of 70% ethanol to the supernatant and mix well by pipetting. Do not centrifuge at this step.
- 4. Transfer 750  $\mu$ l of the mixture to a Column Type F (mini). If the mixture volume exceeds 750  $\mu$ I, repeat the step 4~5 with the remainder of the sample.
- 5. Centrifuge at  $\geq 10,000 \times g$  for 1 min at room temperature. Discard the passthrough and the mini column back into the collection tube.

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

- 6. Add 350 µl of Buffer RW and centrifuge at ≥10,000 x g for 30 sec. Discard the pass-through and reinsert the mini column back into the collection tube.
- 7. Add 70  $\mu$ I of DNase I reaction mixture on to the center of the mini column membrane and incubate for 10 min at room temperature.

To make DNase I reaction mixture, add 2  $\mu$ I of DNase I solution to 70  $\mu$ I of Buffer DRB per isolation. And keep it on ice to protect the activity of DNase I until use.

- 8. Add 350  $\mu$ l of Buffer RW and centrifuge at  $\geq$  10,000 x g for 30 sec. Discard the pass-through and reinsert the mini column back into the collection tube.
- 9. Add 500  $\mu$ l of Buffer RSW and centrifuge at  $\geq$  10,000 x g for 30 sec. Discard the pass-through and reinsert the mini column back into the collection tube.

- 10. Add 500  $\mu$ l of Buffer RSW again and centrifuge at  $\geq$ 10,000 x g for 30 sec. Discard the pass-through and reinsert the mini column back into the collection tube.
- 11. Centrifuge at full speed (>13,000 x g) for 1 min to remove residual wash buffer. Place the mini column into a fresh 1.5 ml microcentrifuge tube (provided).

Residual ethanol may interfere with downstream applications. Care must be taken at this step for eliminating the carryover of Buffer RSW.

12. Add 50  $\mu$ l of of Nuclease-free water to the center of the membrane in the mini column. Incubate at the room temperature for 1 min.

Elution volume can be adjusted according to an experiment's purpose. Using the eluent volume of less than 50  $\mu$ l will be decrease the total RNA yield but increase the concentration of RNA. But for effective elution of RNA, more than 30  $\mu$ l of the eluent should be applied, because too lower volume of eluent cannot soak the membrane completely.

13. Centrifuge at  $\geq$ 10,000 x g for 1 min at room temperature.

Purified RNA can be stored at  $4^{\circ}$ C for immediate analysis and can be stored at  $-70^{\circ}$ C for long-term storage.

## Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low yield	Sample not homogenized completely	Insufficient disruption can lead to decrease in yield of total RNA. Insufficient disruption of samples may attributed to several reasons; - Insufficient mixing with Buffer RAL - Too much samples in the starting sample - Poor disruption of sample Confirm complete homogenization of the sample in Buffer RAL.
	Too much starting sample	Reduce the amount of starting sample. Especially for tissue sample, obey the correct amount of starting sample as indicated in the protocol.
	Poor quality of starting material	Process the sample immediately after harvest from animal if possible. Freeze the harvested tissue rapidly in liquid nitrogen and store at -70°C for later use.
	Culture media not completely removed	Remaining culture media affect lysis efficiency and binding condition. Discard the remaining culture media as completely as possible.
Column	Sample not homogenized completely	Insufficient disruption can lead to decrease in yield of total RNA. Insufficient disruption of samples may attributed to several reasons; - Insufficient mixing with Buffer RAL - Too much samples in the starting sample - Poor disruption of sample Confirm complete homogenization of the sample in Buffer RAL.
	Too much starting sample	Reduce the amount of starting sample. Especially for tissue sample, obey the correct amount of starting sample as indicated in the protocol.

Facts	Possible Causes	Suggestions
RNA degradation	Sample manipulated too much before process	Process the tissue sample immediately after harvest from animal. For cultured cells sample, minimize washing steps in cell harvest.
	Improper storage of RNA	Store isolated RNA at -70°C, Do not store at -20°C.
	Use of RNase- contaminated reagents or disposables	Make sure to use RNase-free products only.
	Incorrect treatment of β-mercaptoethanol during lysis	Ensure that the correct volume of $\beta$ -mercaptoethanol is used in lysis buffer for RNase elimination. The effective concentration of $\beta$ -mercaptoethanol is 1% of the Buffer RAL.
DNA contamination	Incorrect treatment of DNase I reaction mixture	For sufficient enzymatic reaction, add DNase I reaction mixture onto the center of the membrane in the mini column.
Eluate does not perform well in downstream application	Residual ethanol remains in eluate	To remove any residual ethanol included in Buffer RSW from mini column membrane, additional centrifuge step should be performed certainly (step 13). If the carryover of ethanol still remains in the mini column membrane, perform step 13 again until completely done.

# Purification of total RNA without DNase I treatment

Appendix I describes how to purify the total RNA without DNase I treatment from the samples. If DNase I treatment is not required, follow this procedure.

I. Prepare the lysate using an appropriate sample preparation protocol as follows.

### For Cell samples

Add harvested cell samples into a 1.5 ml microcentrifuge tube (not provided) and add 350 or 700  $\mu$ l of Buffer RAL (Refer to Table 3).

Then, homogenize the cell sample by pipetting or microhomogenizer.

Refer to Table 3 for suitable volume of Buffer RAL according to cell amount.

### For Tissue samples

Prepare tissue sample in a tube and add 350 or 700  $\mu$ l of Buffer RAL (including 1%  $\beta$ -mercaptoethanol). Then, homogenize the tissue sample by using an appropriate homogenizer.

Centrifuge at  $\geq$  10,000 x g for 2 min at room temperature and transfer the supernatant to a fresh 1.5 ml microcentrifuge tube.

 $\beta$ -mercaptoethanol must be added to Buffer RAL for homogenizing the tissue samples.

Add 1% β-mercaptoethanol to Buffer RAL proportionally.

(ex. Add 10  $\mu$ l of  $\beta$ -mercaptoethanol to 1 ml of Buffer RAL)

Refer to Table 3 for suitable volume of Buffer RAL according to tissue amount.

Table 3. Volume of Buffer RAL for homogenizing samples.

Amount of starting material	Volume of Buffer RAL
$\sim$ 5 x 10 $^6$ cells or 20 mg tissues	$350\mu$ l
$5 \times 10^6 \sim 1 \times 10^7$ cells or $20 \sim 30$ mg tissues	$700\mu$ l

2. Add I volume (usually 350 or 700  $\mu$ I) of 70% ethanol to the lysate and mix well by pipetting. Do not centrifuge.

- 3. Transfer the mixture to a Column Type F (mini).
- 4. Centrifuge ≥10,000 x g for I min at room temperature. Discard the pass-through and reinsert the mini column back into the same tube.

If the mixture volume exceeds 750  $\mu$ I, repeat step 3 $\sim$ 4 with the remainder of the sample.

- 5. Add 700  $\mu$ l of Buffer RW and centrifuge at  $\geq$ 10,000 x g for 30 sec. Discard the pass-through and reinsert the mini column back into the collection tube.
- 6. Add 500  $\mu$ l of Buffer RSW and centrifuge at  $\geq$  10,000 x g for 30 sec. Discard the pass-through and reinsert the mini column back into the collection tube.
- Add 500 µl of Buffer RSW again and centrifuge at ≥10,000 x g for 30 sec.
   Discard the pass-through and reinsert the mini column back into the collection tube.
- 8. Centrifuge at full speed (>13,000 x g) for 1 min to remove residual wash buffer. Place the mini column into a fresh 1.5 ml microcentrifuge tube (provided).
- 9. Add 50  $\mu$ I of Nuclease-free water to the center of the membrane in the mini column. Incubate at the room temperature for I min.

Elution volume can be adjusted according to an experiment's purpose. Using the eluent volume of less than 50  $\mu$ l will be decrease the total RNA yield but increase the concentration of RNA. But for effective elution of RNA, more than 30  $\mu$ l of eluent should be applied, because too lower volume of eluent cannot soak the membrane completely.

10. Centrifuge at  $\geq$  10,000 x g for 1 min at room temperature.

Purified RNA can be stored at  $4^{\circ}$ C for immediate analysis and can be stored at  $-70^{\circ}$ C for long-term storage.

## **APPENDIX 2**

### **DNase I treatment in RNA eluate**

Appendix 2 describes how to use the DNase I (included in this kit) to eliminate contaminating genomic DNA in RNA eluate. For high DNA contents samples, this procedure is more efficient than on-column DNase I treatment and we are strongly recommended for those samples.

- 1. The mixture as below in a 1.5 ml microcentrifuge tube.
  - 50 μl RNA eluate
  - 5 μl Buffer DRB
  - I µI DNase I solution
- 2. Incubate the mixture for 10 min at room temperature.
- 3. Add I  $\mu$ I of 0.25 M EDTA per 50  $\mu$ I eluate.
- 4. Inactivate DNase I enzyme at 75°C for 10 min.

<sup>\*</sup> For efficient and convenient method of clean-up the DNase I treated-RNA eluate, refer to Appendix 3 or use Riboclear<sup>TM</sup> Plus (Cat. No. 313-150).

## **APPENDIX 3**

## Clean-Up of total RNA

Appendix 3 provides a convenient method for clean-up of total RNA previously purified by other methods.

### Before experiment

- A maximum of 100  $\mu$ g RNA/100  $\mu$ l can be cleaned up by this protocol.
- In case that DNase I treatment step is needed, refer to Appendix 2.
- I. Adjust the sample to 100  $\mu$ l with Nuclease-free water, add 350  $\mu$ l of Buffer RAL and mix thoroughly.
- 2. Add 250  $\mu$ l of absolute ethanol to the sample and mix well by pipetting. Do not centrifuge.
- 3. Transfer the sample to a Column Type F (mini) and centrifuge at ≥10,000 x g for 30 sec. Discard the pass-through and reinsert the mini column back into the collection tube.
- 4. Add 500  $\mu$ l of Buffer RSW and centrifuge at  $\geq$  10,000 x g for 30 sec. Discard the pass-through and reinsert the mini column back into the collection tube.
- 5. Add 500  $\mu$ l of Buffer RSW again and centrifuge at  $\geq$ 10,000 x g for 30 sec. Discard the pass-through and reinsert the mini column back into the collection tube.
- 6. Centrifuge at full speed ( $>13,000 \times g$ ) for I min to remove residual wash buffer. Place the mini column into a fresh 1.5 ml microcentrifuge tube.
  - Residual ethanol may interfere with downstream applications. Care must be taken at this step for eliminating the carryover of Buffer RSW.

### 7. Add 50 $\mu$ l of Nuclease-free water to the center of the membrane in the mini column. Incubate at the room temperature for I min.

Elution volume can be adjusted according to an experiment's purpose. Using the eluent volume less than 50  $\mu$ l will be decrease the total RNA yield but increase the concentration of RNA. But for effective elution of RNA, more than 30  $\mu$ l of eluent should be applied, because too lower volume of eluent cannot soak the membrane completely.

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

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

## **Ordering Information**

Products	Scale	Size	Cat. No.	Туре	Products	Scale	Size	Cat. No.	Туре	
GeneAll® <b>Hybri</b> d	<b>I-Q<sup>TM</sup></b> fo	r rapid pi	reparation of	plasmid DNA	GeneAll® Exgene	tm for is	olation o	f total DNA		
Plasmid Rapidprep	mini	50 200	100-150	spin		mini	100 250	105-101	spin / vacuum	
					Blood SV	Midi	26	105-226	spin /	
GeneAll® <i>Expre</i> p	for p	for preparation of plasmid DNA		DNA		i iidi	100	105-201	vacuum	
r	mini	50	101-150	spin /		MAXI	26	105-310	spin / vacuum	
Diseased CV/		200	101-102	vacuum			100	103-326		
Plasmid SV	N 41 11	26	101-226	spin /		mini	250	106-101	spin / vacuum	
	Midi	50	101-250	vacuum	Cell SV		10	106-132		
		100	101-201			MAXI	26		spin / vacuum	
GeneAll <sup>®</sup>				: 1011				106-326		
for prepa	iration of		ion-grade pla	ismid DINA		mini	250	108-101	spin / vacuum	
	mini	50	111-150	spin /						
Plasmid LE		200	111-102	vacuum	Clinic SV	Midi	26	108-226	spin /	
(Low Endotoxin)	Midi	26	111-226	spin /			100	108-201	vacuum	
		100	111-201	vacuum		MAXI	10	108-310	spin /	
Plasmid EF	Midi	20	121-220	spin			26	108-326	vacuum	
(Endotoxin Free)		100 12	121-201	'	Genomic DNA micro	)	50	118-050	spin	
_						mini	100	117-101	spin /	
GeneAll® <i>Expin</i> ™	<b>m</b> for pur	ification (	of fragment D	NA			250	117-152	vacuum	
Gel SV	mini	50	102-150	spin /	Plant SV	Midi	26	117-226	spin /	
Gei 3V	1111111	200	102-102	vacuum			100	117-201	vacuum	
PCR SV	noini	50	103-150	spin /		MAXI	10	117-310	spin /	
rcn sv	mini	200	103-102	vacuum			26	117-326	vacuum	
Classilla CV		50	113-150	spin /	Soil DNA mini	mini	50	114-150	spin	
CleanUp SV	mini	200	113-102	vacuum	Stool DNA mini	mini	50	115-150	spin	
6 1 60		50	112-150	spin /	Stool-Bead DNA mini	mini	50	115-151	spin	
Combo GP	mini	200	112-102	vacuum	Viral DNA/RNA	mini	50	128-150	spin	
					FFPE Tissue DNA	mini	50	138-150	spin	
GeneAll® <i>Exgen</i> e	e <sup>TM</sup> for is	olation o	f total DNA		TITE TISSUE DIVA	1111111	250	138-152	spiri	
	mini	100 104-101		spin /		for	isolation	of total DNA		
	mini	250	104-152	vacuum	GeneAll® <b>GenEx</b> 1	<b>w</b> ith	nout spin	column		
T 01	- NA: 1:	26	104-226	spin /		Sx	100	220-101	- solution	
Tissue SV	Midi	100	104-201	vacuum	GenEx <sup>™</sup> Blood		500	220-105		
		10	104-310	spin /	-	Lx	100	220-301	solution	
	MAXI	26	104-326	vacuum	-		100	221-101	1	
-		100	109-101	spin /	GenEx <sup>™</sup> Cell	Sx	500	221-105	solution	
	mini	250	109-152	vacuum	-	Lx	100	221-301	solution	
		26	109-226	spin /	-	-	100	222-101	1.2	
Tissue Plus SV	Midi	100	109-201	vacuum	GenEx <sup>™</sup> Tissue	Sx	500	222-105	- solution	
		10	109-310	spin /	<del></del>	Lx	100	222-301	solution	
	MAXI	26	109-326	vacuum						
			. 5, 520							

Products	Scale	Size	Cat. No.	Туре
GeneAll® GenEx	TAA '	solation out spin	of total DNA column	
	Sx	100	227-101	
GenEx <sup>™</sup> Plant	Mx	100	227-201	solution
	Lx	100	227-301	
	Sx	100	228-101	
GenEx <sup>™</sup> Plant Plus	Mx	50	228-250	solution
-	Lx	20	228-320	

## GeneAll® *DirEx*™ series

1 FF	-1		
DirEx <sup>™</sup>	100	250-101	solution
DirEx <sup>™</sup> Fast-Tissue	96 T	260-011	solution
DirEx <sup>™</sup> Fast-Cultured cell	96 T	260-021	solution
DirEx <sup>™</sup> Fast-Whole blood	96 T	260-03 I	solution
DirEx <sup>™</sup> Fast-Blood stain	96 T	260-041	solution
DirEx <sup>™</sup> Fast-Hair	96 T	260-051	solution
DirEx <sup>™</sup> Fast-Buccal swab	96 T	260-061	solution
DirEx <sup>™</sup> Fast-Cigarette	96 T	260-071	solution

### GeneAll® RNA series for preperation of total RNA

RiboEx <sup>™</sup>	mini	100	301-001	solution
		200	301-002	SOIULION
Hybrid-R <sup>™</sup>	mini	100	305-101	spin
$\overline{\text{Hybrid-R}^{\text{TM}} \text{Blood RNA}}$	mini	50	315-150	spin
Hybrid-R <sup>™</sup> miRNA	mini	50	325-150	spin
RiboEx <sup>™</sup> LS	:_:	100	302-001	solution
KIDOEX LS	mini	200	302-002	solution
Riboclear™	mini	50	303-150	spin
Riboclear <sup>™</sup> Plus	mini	50	313-150	spin
Ribospin <sup>™</sup>	mini	50	304-150	spin
	mini	50	314-150	spin
Ribospin <sup>™</sup> II		300	314-103	
Ribospin ™ vRD	mini	50	302-150	spin
Ribospin <sup>™</sup> vRD Plus	mini	50	312-150	spin
Ribospin <sup>™</sup> vRD II	mini	50	322-150	spin
Ribospin <sup>™</sup> Plant	mini	50	307-150	spin
Ribospin <sup>™</sup> Seed/Fruit	mini	50	317-150	spin
Ribospin <sup>™</sup>		50	314-150	!-
Pathogen/TNA	mini	250	314-152	spin
$Allspin^{TM}$	mini	50	306-150	spin
$RiboSaver^{TM}$	mini	100	351-001	solution

Products	Scale	Size	Cat. No.	Туре	
GeneAll® AmpONE <sup>TM</sup> for PCR amplification					
		250 U	501-025		
Taq DNA polymerase		500 U	501-050	(2.5 U/µI)	
		I,000 U	501-100		
T Di-	$20 \mu$ l x 96 tubes		526-200	1-41	
Taq Premix	50 μl x 96 tubes		526-500	solution	

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

T M+	0.5 ml x 2 tubes	541-010	solution
Taq Master mix	0.5 ml x 10 tubes	541-050	solution

### GeneAll® HyperScript<sup>TM</sup> for Reverse Transcription

Reverse Transcripta	se 10,000 U	601-100	solution
RT Master mix	0.5 ml x 2 tubes	601-710	solution
One-step RT-PCR Master mix	0.5 ml x 2 tubes	602-110	solution
One-step RT-PCR Premix	$20 \mu$ l x 96 tubes	602-102	solution

### $\textbf{GeneAll}^{\textbf{®}}\,\textbf{RealAmp}^{\textbf{TM}}\,\,\textit{for qPCR amplification}$

SYBR qPCR Master	200 rxn	2 ml	801-020	solution
mix (2X, Low ROX)	500 rxn	5 ml	801-050	solution
SYBR qPCR Master	200 rxn	2 ml	801-021	solution
$\operatorname{mix}\left(2X,\operatorname{High}\operatorname{ROX}\right)$	500 rxn	5 ml	801-051	Solution

### GeneAll® Protein series

ProtinEx <sup>™</sup> Animal cell/tissue	100 ml	701-001	solution
PAGESTA <sup>™</sup> Reducing 5X SDS-PAGE Sample Buffer	I ml x 10 tubes	751-001	solution

Products	Size	Cat. No.	Туре	
GeneAll® GENT; TM 32 Newly designed automated extraction system				
Automatic extraction equipr	nent	GTI032A	system	
Genomic DNA	48	901-048A	tube	
Genomic DINA	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	
	48	904-048A	tube	
Plant DNA/RNA	96	904-096A	plate	
LMO	48	906-048A	tube	
LMO	96	906-096A	plate	
Fecal DNA/RNA	48	913-048A	tube	
	96	913-096A	plate	

GeneAll® AllEx®64 Compact yet Comprehensive automated extraction system				
Automatic extraction equipmer	nt	AEX064	system	
C : DNU	48	931-048	tube	
Genomic DNA	96	931-096	plate	
Versal DNIA /DNIA	48	934-048	tube	
Viral DNA/RNA	96	934-096	plate	
DII DAIA	48	935-048	tube	
Blood DNA	96	935-096	plate	
Plant DNA/RNA	48	937-048	tube	
PIANT DINAYRINA	96	937-096	plate	
Fecal DNA/RNA	48	948-048	tube	
	96	948-096	plate	
Forensic	48	936-048	tube	
Toronsic	96	936-096	plate	

## NOTE —

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#### **GENEALL BIOTECHNOLOGY CO., LTD.**

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