Ver I.I HB 3410

RibospinTM 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.

Contact Information

www.geneall.com

Tel: 82-2-407-0096

Fax: 82-2-407-0779

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

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

Visit GeneAll® Community

www.geneall.com

www.geneall.co.kr

Trademarks

Hybrid- Q^{TM} , ExprepTM, ExfectionTM, ExpinTM, ExgeneTM, GenExTM, DirExTM, Hybrid-RTM, RiboExTM, RiboSaverTM, EzClearTM, EzSepTM, EzPureTM, EzPassTM, AmpONETM, AmpMasterTM, RealAmpTM, HyperScriptTM, ProtinExTM, PAGESTATM, STEADiTM, GENTiTM are trademarks of GeneAll Biotechnology Co., Ltd.

© 2019 GeneAll Biotechnology, all rights reserved.

This protocol handbook is included in :

GeneAll® RibospinTM II (314-150, 314-103)

Visit www.geneall.com or www.geneall.co.kr for FAQ, Q&A and more information.

Animal Cell

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

Lyse





- 2. Add 350 μ l (700 μ 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





DNase I treatment

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

10. Add 350 μ l Buffer RW

12. Add 500 μ l Buffer RSW

14. Repeat step 12~13



II. Centrifuge at \geq 10,000 x g for 30 sec

13. Centrifuge at \geq 10,000 x g for 30 sec

Wash







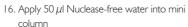


Elute



Pure RNA





15. Additional centrifuge at $> 13,000 \times g$ for 1 min

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



- II. Add 350 μ I Buffer RW
- 12. Centrifuge at \geq 10,000 x g for 30 sec
- 13. Add 500 μ 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 μ 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

INDEX

Brief Protocol	03
Index	05
Kit Contents	06
Materials Not Provided	
Product Specifications	
Quality Control	07
Storage Conditions	
Safety Information	
Product Disclaimer	
Preventing RNase Contamination	08
Preparation of DNase I Solution	
Product Description	09
Protocol I	10
Total RNA from animal cell	
Protocol 2	13
Total RNA from animal tissue	
Troubleshooting Guide	16
APPENDIX I	18
+	
Purification of total RNA without DNase I treatment	
APPENDIX 2	20
	20
APPENDIX 2 DNase I treatment in RNA eluate	
APPENDIX 2 DNase I treatment in RNA eluate APPENDIX 3	20 21
APPENDIX 2 DNase I treatment in RNA eluate	
APPENDIX 2 DNase I treatment in RNA eluate APPENDIX 3	

Kit Contents

Cat. No.	314-150	314-103	
Туре	mini	mini	Storage
Components	Qua	ntity	
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	
Protocol handbook	1	1	

^{*} 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	1×10^7 cells or 30 mg tissue/prep				
Preparation time	≥30 min				
Maximum loading volume of mini column	750 <i>μ</i> Ι				
Minimum elution volume	30 <i>μ</i> Ι				
Maximum binding capacity	500 μg				

^{*} Contains sodium azide as a preservative.

^{**} 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 GeneAll® 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 GeneAll® RibospinTM 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.

GeneAll® RibospinTM II is guaranteed until the expiration date printed on the product box.

Safety Information

The buffers included in the GeneAll® Ribospin™ 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

GeneAll[®] Ribospin[™] 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 μ 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 μ I DNase I solution is required.

Product Description

GeneAll[®] Ribospin[™] II is devised to purify RNA from cultured cells or animal tissues (~1 x 10⁷ 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 GeneAll® Ribospin™ 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 longterm storage.

We strongly recommend reading the procedure to using GeneAll $^{\mathbb{R}}$ Ribospin $^{\mathsf{TM}}$ II.

9

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 μ l of DNase I solution with 70 μ 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 5×10^6 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 5×10^6 cells by centrifugation at low speed (below $800 \times g$) for 5 minutes, and then discard the culture medium.

2. Add 350 or 700 μ 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 μ 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 µl
$5 \times 10^6 \sim 1 \times 10^7$ cells	700 μl

^{*} Do not wash the cells before lysing with Buffer RAL as this may cause mRNA degradation.

- 3. Add I volume (usually 350 or 700 μ I) of 70% ethanol to the lysate and mix well by pipetting. Do not centrifuge.
- 4. Transfer 750 μ l of the mixture to a Column Type F (mini). If the mixture volume exceeds 750 μ 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 μ 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 μ 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 μ I of DNase I solution to 70 μ I of Buffer DRB per isolation. And keep it on ice to protect the activity of DNase I until use.
- 8. Add 350 μ 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 μ 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 μ 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 μ 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 μ l will be decrease the total RNA yield but increase the concentration of RNA. But for effective elution of RNA, more than 30 μ 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[™].
- In case that the preserved sample in RNA stabilization solution like RiboSaver[™], the stabilization solution should be discarded completely.
- Make 1% β -mercaptoethanol (ex, 10 μ 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 μ l of DNase I solution with 70 μ 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 1% β-mercaptoethanol)		
~20 mg	350 µl		
20 mg~30 mg	700μ 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 μ l of Buffer RAL (Refer to Table 2) (including 1% β-mercaptoethanol) and pulse-vortex for 30 sec.

- Ib. Homogenize up to 20 mg of the tissue sample in 350 or 700 μ 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 μ l of Buffer RAL (including 1% β -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 μ I) of 70% ethanol to the supernatant and mix well by pipetting. Do not centrifuge at this step.
- 4. Transfer 750 μ l of the mixture to a Column Type F (mini). If the mixture volume exceeds 750 μ 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 μ 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 μ I of DNase I solution to 70 μ I of Buffer DRB per isolation. And keep it on ice to protect the activity of DNase I until use.

- 8. Add 350 μ 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 μ 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 μ 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 μ 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 μ l will be decrease the total RNA yield but increase the concentration of RNA. But for effective elution of RNA, more than 30 μ 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 and can be stored at -70° 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 clogging	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 β -mercaptoethanol is used in lysis buffer for RNase elimination. The effective concentration of $\beta\text{-mercaptoethanol}$ 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 μ 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 μ l of Buffer RAL (including 1% β -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.

 β -mercaptoethanol must be added to Buffer RAL for homogenizing the tissue samples.

Add 1% β-mercaptoethanol to Buffer RAL proportionally.

(ex. Add 10 μ l of β -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 <i>µ</i> l
$5 \times 10^6 \sim 1 \times 10^7$ cells or $20 \sim 30$ mg tissues	700μ l

2. Add I volume (usually 350 or 700 μ 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 μ I, repeat step 3 \sim 4 with the remainder of the sample.

- 5. Add 700 μ 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 μ 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 μ 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 μ l will be decrease the total RNA yield but increase the concentration of RNA. But for effective elution of RNA, more than 30 μ 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° C for immediate analysis and can be stored at -70° 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 μ I of 0.25 M EDTA per 50 μ I eluate.
- 4. Inactivate DNase I enzyme at 75°C for 10 min.

^{*} For efficient and convenient method of clean-up the DNase I treated-RNA eluate, refer to Appendix 3 or use RiboclearTM 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 μ g RNA/100 μ 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 μ l with Nuclease-free water, add 350 μ l of Buffer RAL and mix thoroughly.
- 2. Add 250 μ 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 μ 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 μ 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 μ 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 μ l will be decrease the total RNA yield but increase the concentration of RNA. But for effective elution of RNA, more than 30 μ 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® <i>Hybrid</i>	-Q[™] fo	r rapid p	reparation of	plasmid DNA	GeneAll® Exgen	e TM for is	olation o	f total DNA	
Plasmid Rapidprep		50	100-150				100	105-101	spin /
	mini	200	100-102	spin		mini	250	105-152	vacuum
				DI 4 CV	M: J:	26	105-226	spin /	
GeneAll® <i>Exprep</i>	TM for p	reparatio	n of plasmid DNA		Blood SV	Midi	100	105-201	vacuum
		50	101-150	spin /		MAXI	10	105-310	spin /
	mini	200	101-102	vacuum		MAXI	26	105-326	vacuum
D		26	101-226				100	106-101	spin /
Plasmid SV	Midi	50	101-250	spin /	Cell SV	mini	250	106-152	vacuum
		100	101-201	vacuum	Cell 3V	MANI	10	106-310	spin /
GeneAll® <i>Exfecti</i>	on TM					MAXI	26	106-326	vacuum
for prepa	ration of	transfect	ion-grade pla	smid DNA			100	108-101	spin /
	<u> </u>	50	111-150	spin /		mini	250	108-152	vacuum
Plasmid LE	mini	200	111-102	vacuum	CI: : C) /	- N4: 1:	26	108-226	spin /
(Low Endotoxin)		26	111-226	spin /	Clinic SV	Midi	100	108-201	vacuum
	Midi	100	111-201	vacuum			10	108-310	spin /
Plasmid EF		20	121-220			MAXI	26	108-326	vacuum
(Endotoxin Free)	Midi	100	100 121-201 spin	Genomic DNA micr	·o	50	118-050	spin	
,							100	117-101	spin /
GeneAll® <i>Expin</i> ™	v for bur	ification (of fragment D	NA		mini	250	117-152	vacuum
General Expin 101 po	1 F	50	102-150	spin / Plant SV		26	117-226	spin /	
Gel SV mini	mini	200	102-102		Plant SV	Midi	100	117-201	vacuum
		50	103-150	spin /			10	117-310	spin /
PCR SV	mini	200	103-102	vacuum		MAXI	26	117-326	vacuum
		50	113-150		Soil DNA mini	mini	50	114-150	spin
CleanUp SV	mini	200	113-102	spin / vacuum	Stool DNA mini	mini	50	115-150	spin
		50	112-150		Viral DNA / RNA	mini	50	128-150	spin
Combo GP	mini	200	112-130	spin / vacuum			50	138-150	
				vacuum	FFPE Tissue DNA	mini	250	138-152	spin
GeneAll® Exgene	for is				GeneAll® GenE x	TM for iso	lation of	total DNA wit	hout spin
	mini	100	104-101	spin /		•	100	220-101	· ·
		250	104-152	vacuum	GenEx [™] Blood	Sx	500	220-101	solution
Tissue SV	Midi	26	104-226	spin /	SCIEX BIOOG	Lx	100	220-103	solution
		100	104-201	vacuum	-	L^	100	221-101	JOIULIOI
	MAXI	10	104-310	spin /	GenEx [™] Cell	Sx	500	221-101	solution
		26	104-326	vacuum	GUILA CEII		100	221-103	solution
	mini	100	109-101	spin /		LX	100		JOIULIOI
		250	109-152	vacuum	GenEx [™] Tissue	Sx	500	222-101	solution
Tissue plus! SV	Midi	26	109-226	spin /	GenEx''' lissue		100		columbia :
rissuc pius: 3v	ı ildi	100	109-201	vacuum		LX	100	222-301	solution
	MAXI	10	109-310	spin /					
N		26							

Products	Scale	Size	Cat. No.	Туре
GeneAll® GenEx	TM for iso	olation of	total DNA	
	Sx	100	227-101	
GenEx [™] Plant	Mx	100	227-201	solution
	Lx	100	227-301	
	Sx	100	228-101	
GenEx [™] Plant plus!	Mx	50	228-250	solution
	Lx	20	228-320	

GeneAll® *DirEx™* series

for preperation 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	260-03 I	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 preperation of total RNA

	100	301-001	solution
mini	200	301-002	
mini	100	305-101	spin
4 mini	50	315-150	spin
mini	50	325-150	spin
maini	100	302-001	actuation
mini	200	302-002	solution
mini	50	303-150	spin
mini	50	313-150	spin
mini	50	304-150	spin
mini	50	314-150	
	300	314-103	spin
mini	50	302-150	spin
mini	50	312-150	spin
mini	50	322-150	spin
mini	50	307-150	spin
mini	50	317-150	spin
mini	50	306-150	spin
mini	100	351-001	solution
	Amini mini mini mini mini mini mini mini	mini 200 mini 100 Amini 50 mini 50	mini 200 301-002 mini 100 305-101 Amini 50 315-150 mini 50 325-150 mini 200 302-001 mini 50 303-150 mini 50 313-150 mini 50 314-150 mini 50 314-150 mini 50 302-150 mini 50 312-150 mini 50 322-150 mini 50 307-150 mini 50 317-150 mini 50 307-150

Products	Scale	Size	Cat. No.	Туре	
GeneAll® AmpO	NETM for	PCR ar	mplification		
Taq DNA polymerase		250 U	501-025		
		500 U	501-050	(2.5 U/ µℓ)	
		,000 U	501-100		
	96 tubes-	20 µl	526-200		
Taq Premix	70 tubes		526-500	solution	

GeneAll® AmpMasterTM for PCR amplification

To a Mastan min	0.5 ml x 2 tubes	541-010	solution
Taq Master mix	0.5 ml x 10 tubes	541-050	solution

GeneAll[®] HyperScript[™] for Reverse Transcription

		,	
Reverse Transcripta	ase 10,000 U	601-100	solution
RT Master mix	$0.5~\mathrm{ml} \times 2~\mathrm{tubes}$	601-710	solution
One-step RT-PCR Master mix	$0.5~\mathrm{ml} \times 2~\mathrm{tubes}$	602-110	solution
One-step RT-PCR Premix	96 tubes, 20 μl	602-102	solution

GeneAll® RealAmp[™] for qPCR amplification

SYBR qPCR Master	200 rxn	20 µl	801-020	solution
mix (2X, Low ROX)	500 rxn	20 μl	801-050	SOIULION
SYBR qPCR Master	200 rxn	20 μl	801-021	solution
mix (2X, High ROX)	500 rxn	20 μl	801-051	SOlution

Products	Size	Cat. No.	Туре
1100000	OILC	Out. 1 10.	1770

GeneAll® Protein series

ProtinEx [™] Animal cell / tissu	e 100 ml	701-001	solution
PAGESTA [™] Reducing 5X SDS-PAGE Sample Buffer	I ml × 10 tubes	751-001	solution

GeneAll $^{ ext{@}}$ STEAD $\dot{\iota}^{ ext{ iny m}}$ for automatic nucleic acid puritication

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 $^{\circledast}$ GENT i^{TM} $^{\mathsf{SE}}$ Ultimately flexible automatic extraction system

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

NOTE —

NOTE —



www.geneall.com

GeneAll Bldg., 303-7 Dongnam-ro, Songpa-gu, Seoul, South Korea 05729

E-mail: sales@geneall.com

Tel: 82-2-407-0096 Fax: 82-2-407-0779