

# GeneAll® Riboclear<sup>TM</sup> plus!

#### Kit Contents

Components	Quantity	Storage
DNase I	55 ul	-20 ℃
Buffer MS	30 ml	
Buffer RNW	60 ml	
RNase-free water	15 ml	
DNase I buffer (10 X)	1 ml	Room temperature
Micro column type S (with collection tube)	50	
1.5 ml collection tube	50	

# Product Specifications

Riboclear <sup>™</sup> plus! Specifications				
Туре	Spin			
Maximum amount of starting samples	~ 100 ul			
RNA recovery rate	~ 95%			
Preparation time	~ 17 minutes			
Maximum loading volume	~ 800 ul			
Minimum elution volume	20 ul			
Binding capacity	~ 100 ug			

# Quality Control

Riboclear<sup>TM</sup> plus! is manufactured in strictly clean condition, and its degree of cleanness is monitored periodically. For consistency of product, the quality certification process is carried out from lot to lot thoroughly and only the qualified is approved to be delivered.

### Storage Conditions

Riboclear™ plus! should be stored at room temperature. But prolonged storage at high temperature over 30°C can reduce the performance of the kit. All components are stable for 1 year. Keep out of direct sunlight.

# Precautions

The buffers included in Riboclear<sup>™</sup> plus! contain irritant which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector, and follow standard safety precautions. In case of contact, wash immediately with plenty of water and seek medical advice

Buffer MS contains chaotropes. It can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste.

### ■ Preventing RNase Contamination

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

GeneAll <sup>®</sup> <b>Hybrid-Q</b> ™	for rapid preparation	n of plasmid D	VΔ	GeneAll <sup>®</sup> GenEx <sup>™</sup> for iso.	lation of total DN	Δ	
Products	Format	Size	Cat, No	Products	Format	Size	Cat, N
		50	100-150	GenEx™ C	Sx†	100	
Plasmid Rapidprep Plasmid Rapidprep	mini mini	200	100-100	GenEx™ C GenEx™ C	Sx†	500	221-10 221-10
			100-102	GenEx™ C	Lx <sup>††</sup>	100	221-10
GeneAll <sup>®</sup> Exprep <sup>™</sup> for	r preparation of plas	smid DNA		GenEx™ T	Sx <sup>†</sup>	100	222-10
Plasmid SV	mini	50	101-150	GenEx™ T	Sx†	500	222-10
Plasmid SV	mini	200	101-102	GenEx™ T	Lx <sup>††</sup>	100	222-30
Plasmid SV	mini	1,000	101-111				
Plasmid SV Plasmid SV	Midi Midi	26	101-226	GeneAll <sup>®</sup> DirEx <sup>™</sup> Single to			
Plasmid SV	Midi	50 100	101-250 101-201	DirEx™	Solution	50	250-05
				GeneAll® RNA Series for	r preparation of I	RNA	
GeneAll <sup>®</sup> Exfection™				Hybrid-R™	spin	100	305-10
Plasmid LE	mini	50	111-150	Hybrid-R™ Blood RNA	spin	50	315-15
Plasmid LE	mini	200	111-102	*			
Plasmid LE	Midi	26	111-226	Hybrid-R™ miRNA	spin	50	325-15
Plasmid LE	Midi	100	111-201	RiboEx™	solution	100	301-00
Plasmid EF	Midi	20	121-220	RiboEx™	solution	200	301-00
Plasmid EF	Midi	100	121-201	RiboEx™ LS	solution	100	302-00
GeneAll <sup>®</sup> Expin <sup>™</sup> for p	ourification of fragme	ent DNA		RiboEx <sup>™</sup> LS	solution	200	302-00
				Riboclear™	spin	50	303-15
Gel SV	mini	50	102-150	Riboclear™ plus!	spin	50	313-15
Gel SV	mini	200	102-102	Ribospin™	spin	50	304-15
PCR SV	mini	50	103-150	,			
PCR SV	mini	200	103-102	Ribospin™ vRD	spin	50	302-15
CleanUp SV	mini	50	113-150	Ribospin™ vRD plus!	spin	50	312-15
CleanUp SV	mini	200	113-102	Ribospin™ Plant	spin	50	307-15
Combo GP	mini	50	112-150	Allspin™			
Combo GP	mini	200	112-102		spin	50	306-15
GeneAll <sup>®</sup> Exgene™ fo				GeneAll® AmpONE™ for	PCR amplification	on	
Geneall Exgene to	r isolation of total D	INA		Taq DNA polymerase	(2.5 U/µℓ)	250 U	501-02
Tissue SV (plus!)*	mini	100	104(9)-101	Taq DNA polymerase	(2.5 U/µℓ)	500 U	501-05
Tissue SV (plus!)*	mini	250	104(9)-152	Taq DNA polymerase	(2.5 U/µℓ)	1000 U	501-10
Tissue SV (plus!)**	Midi	26	104(9)-226	α-Tag DNA polymerase	(2.5 U/µℓ)	250 U	502-02
Tissue SV (plus!)**	Midi	100	104(9)-201		(2.5 U/µℓ)	500 U	502-05
Tissue SV (plus!)**	MAXI	10	104(9)-310	$\alpha$ -Taq DNA polymerase	(2.5 U/µℓ)	1000 U	502-10
Tissue SV (plus!)**	MAXI	26	104(9)-326	Pfu DNA polymerase	(2.5 U/µℓ)	250 U	503-02
Blood SV	mini	100	105-101	Pfu DNA polymerase	(2.5 U/µℓ)	500 U	503-0
Blood SV	mini	250	105-152	Pfu DNA polymerase	(2.5 U/µℓ)	1000 U	503-10
Blood SV Blood SV	Midi Midi	26 100	105-226 105-201	HS-Taq DNA polymerase	(2.5 U/µℓ)	250 U	531-02
Blood SV	MAXI	100	105-201	HS-Taq DNA polymerase	(2.5 U/µℓ)	500 U	531-05
Blood SV	MAXI	26	105-326	HS-Taq DNA polymerase	(2.5 U/µℓ)	1000 U	531-10
Cell SV	mini	100	106-101	Clean Taq DNA polymerase	(2.5 U/µℓ)	250 U	551-02
Cell SV	mini	250	106-152	Clean Taq DNA polymerase	(2.5 U/µℓ)	500 U	551-0
Cell SV	MAXI	10	106-310	Clean Taq DNA polymerase	(2.5 U/µℓ)	1000 U	551-1
Cell SV	MAXI	26	106-326	Clean \alpha-Taq DNA polymeras	2511///	250 U	552-0
		100		Clean $\alpha$ -Tag DNA polymeras		500 U	552-0
Clinic SV Clinic SV	mini mini	250	108-101 108-152	Clean $\alpha$ -Taq DNA polymeras	. ,	1000 U	552-1
Clinic SV	Midi	26	108-132				
Clinic SV	Midi	100	108-201	Tag Premix	96 tubes 96 tubes	20 μl	521-2
Clinic SV	MAXI	10	108-310	Taq Premix		50 μl	521-5
Clinic SV	MAXI	26	108-326		96 tubes	20 μℓ	522-2
Genomic DNA micro	micro	50	118-050		96 tubes	50 μl	522-5
				HS-Taq Premix	96 tubes	20 μℓ	525-2
Plant SV	mini	100	117-101	HS-Taq Premix	96 tubes	20 μℓ	525-5
Plant SV	mini Midi	250	117-152	Taq Master mix 0	.5 ml x 2 tubes	1 ml	541-0
Plant SV Plant SV	Midi Midi	26 100	117-226 117-201		5 ml x 10 tubes	5 ml	541-0
Plant SV	MAXI	100	117-201		.5 ml x 2 tubes	1 ml	542-0
Plant SV	MAXI	26	117-316		5 ml x 10 tubes	5 ml	542-0
GMO SV GMO SV	mini mini	50 200	107-150 107-102		.5 ml x 2 tubes	1 ml	545-0
			101-102		5 ml x 10 tubes	5 ml	545-0
GeneAll <sup>®</sup> GenEx <sup>™</sup> for	isolation of total DN	IA		Taq Premix (w/o dye)	96 tubes	50 μℓ	524-2
GenEx™ B	Sx <sup>†</sup>	100	220-101	dNTP mix	2.5 mM each	500 μℓ	509-0
GenEx™ B	Sx <sup>†</sup>	500	220-105	dNTP set	100 mM	1 ml x 4 tube	509-0
GenEx™ B	Lx <sup>††</sup>	100					

- GeneAll® SV Midi / MAXI kits require the centrifuge which has a swinging-bucket rotor and ability of 4,000 ~ 5,000 xg.
- † On the basis of DNA purification from 300  $\mu\ell$  whole blood. †† On the basis of DNA purification from 10 ml whole blood

#### ■ Product description

Riboclear<sup>™</sup> plus! provides a convenient method for DNase I treatment and clean-up of total RNA. Riboclear<sup>™</sup> plus! procedures employed the glassfiber membrane technology for the clean-up of total RNA, instead of conventional alcohol precipitation.

In this Riboclear  $^{\text{TM}}$  plus! kit, especially, contaminated DNA in extracted total RNA can be removed by DNase I treatment prior to starting the procedure of RNA clean-up.

After the step for removal of contaminated DNA, RNA-containing samples mixed with buffer MS are applied to a micro spin column, followed by centrifugation. RNA binds to silica membrane while most of impurities pass through. The membrane is washed by buffer RNW for removal of some molecules bound nonspecifically. At last, pure RNA is eluted by RNase-free water. Riboclear<sup>TM</sup> *plus!* procedure should be performed at room temperature. The eluate should be treated with care because RNA is very sensitive to contaminants, such as RNases, often found on general lab ware and dust. To ensure RNA-stability, it is recommended to store at 4°C for immediate analysis or to freeze at -70°C for long-term storage.

# ■ Protocol of Riboclear<sup>™</sup> plus!

#### <The procedure for removal of contaminated DNA>

1. Prepare the mixture as below in a 1.5 ml tube.

50 ul RNA eluate

5 ul DNase | buffer (10 X)

1 ul DNase I

If the volume of your sample is more than 50 ul, adjust DNase I buffer to the volume of RNA eluate proportionally.

Also, DNase | is sensitive to physical damage.

Therefore, Do NOT vortex vigorously.

- 2. Incubate the mixture at room temperature for 10 minutes.
- Continue with "The procedure of RNA clean-up and concentration"

#### <The procedure of RNA clean-up and concentration>

 Add 5 volumes of buffer MS to 1 volume of the sample and mix thoroughly.

For 50 ul reaction, add 250 ul of Buffer MS.

- \* Do not centrifuge.
- 2. Transfer the mixture to a micro spin column.
- 3. Centrifuge at ≥ 10,000 xg for 30 seconds.

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

If the mixture volume is more than 700 ul, apply the mixture twice; apply 700 ul of the mixture, spin down, discard the pass-through, re-insert empty collection tube, and repeat the step again until all of the mixture has been applied to the micro spin column.

- 4. Apply 700 ul of buffer RNW.
- 5. Centrifuge at ≥ 10,000 xg for 30 seconds.

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

 Centrifuge at ≥ 10,000 xg for an additional 1 minute to remove residual wash buffer.

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

- Transfer the micro spin column to a new 1.5 ml tube (provided).
- Apply 50 ul of RNase-free water to the center of the membrane in the micro spin column. Let it stand for 1 minute, and centrifuge at ≥ 10,000 xg for 1 minute.

To obtain more concentrated RNA solution, apply 20 ul of RNase-free water. The yield can be significantly decreased if the volume of eluent is lower than 20 ul. Purified RNA can be stored at  $4\,^{\circ}\mathrm{C}$  for immediate analysis and stored at  $-70\,^{\circ}\mathrm{C}$  for long term storage.

#### ■ Troubleshooting Guide

Problem	Possible cause	Suggested solution	
Poor quality and yield of RNA	Incorrect procedure	Buffer MS and samples should be mixed completely. Do not centrifuge after mix.	
	Improper storage of kit	Store kit components at room temperature. Storage at low temperature may cause salt precipitation.  Keep bottles tightly closed in order to avoid evaporation or contamination.	
	RNase-free water applied incorrectly	Ensure that RNase-free water is applied to the center of membrane.	
	Too much volume of RNase-free water	Reduce the volume of eluent.	
Degradation of RNA	Contamination of RNase	RNase can be introduced during use. Be certain not to introduce any RNases during the procedure or later handling. Keep tubes closed whenever possible during the preparation.	
	Improper storage of RNA	Store isolated RNA at -70°C, Do not store at -20°C.	
Genomic DNA contamination	Starting sample has high DNA mass	At step 1, 1 ul of DNase I can be used for upto 25 ug of DNA contaminants.  Increase the DNase I upto 2 ul or decrease the starting sample down to 50 ul.	
	DNase I not active	For prolonged activity, aliquot the DNase I into small portion. Do not freezing and thawing the aliquots several times.	
RNA does not perform well in downstream application	Residual ethanol remains in eluate	To remove any residual ethanol included in buffer RNW from micro spin column membrane, centrifuge again for complete removal of ethanol (step 6).	

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