Ver 2.0

Hybrid-R™ Blood RNA

BLOOD TOTAL RNA PURIFICATION HANDBOOK

CE IVD

GeneAll

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

Trademarks

Hybrid-Q™, Exprep™, Exfection™, Expin™, Exgene™, *GenEx™*, DirEx™, Hybrid-R™, RiboEx™, Ribospin™, Riboclear™, Allspin™, RiboSaver™, EzClear™, EzSep™, EzPure™, EzPass™, AmpONE™, AmpMaster™, RealAmp™, HyperScript™, ProtinEx™, PAGESTA™, STEADi™, GENTi™, SolMate™ are trademarks of GeneAll Biotechnology Co., Ltd.

This protocol handbook is included in :

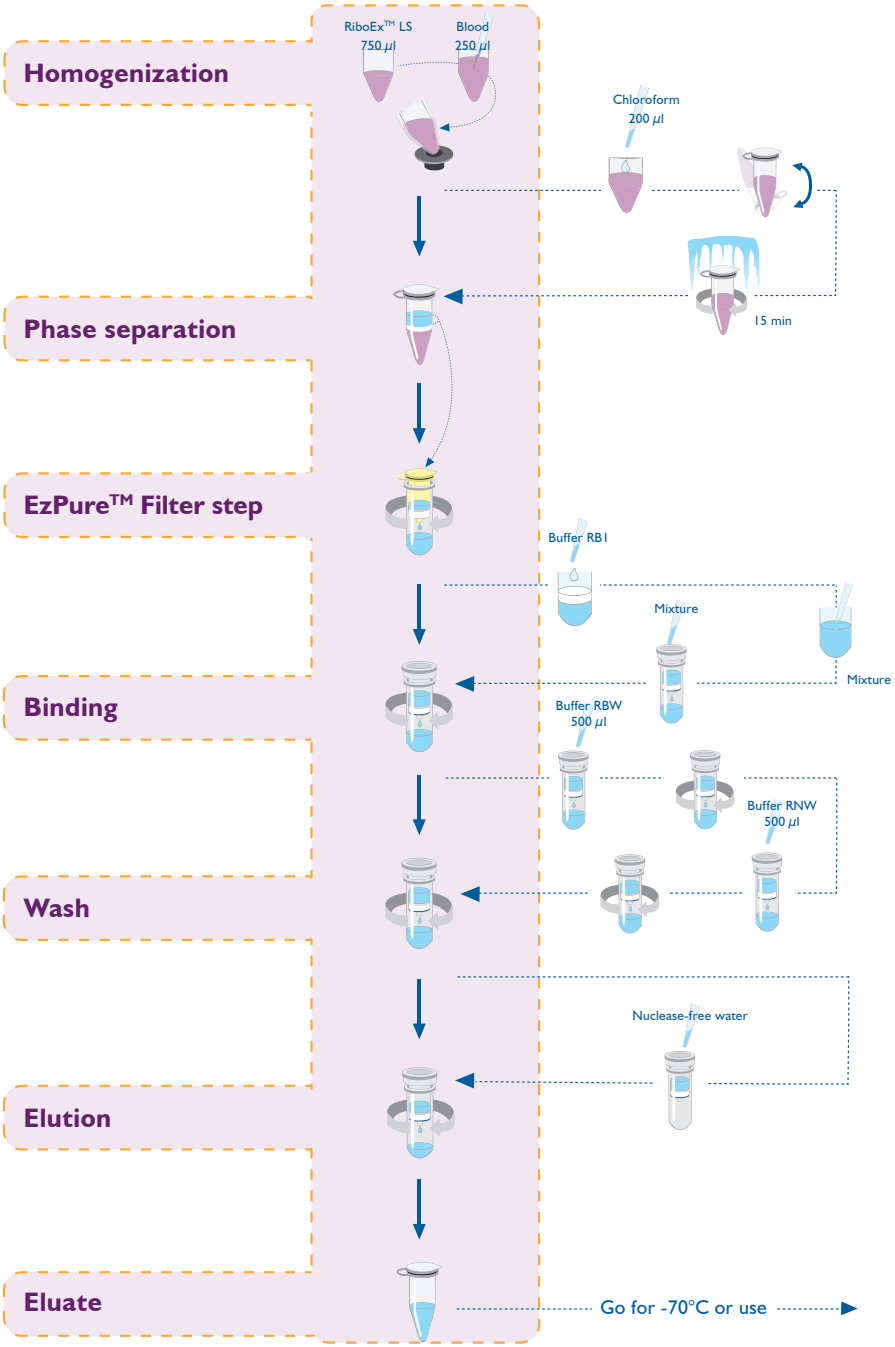
GeneAll® Hybrid-R™ Blood RNA (315-150)

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

Brief Protocol

Homogenization	Lyse ~250 µl whole blood / 750 µl RiboEx™ LS. Incubate the lysate for 2 min at RT.
Phase separation	Add 200 µl chloroform. Incubate the mixture for 2 min at RT. Centrifuge at 12,000 x g for 15 min at 4°C.
EzPure™ Filter step	Transfer the aqueous phase to a EzPure™ Filter and centrifuge at ≥10,000 x g for 30 sec.
Binding	Add 2 volume of Buffer RB1 to the collection tube including passed-through and mix thoroughly by pipetting. Transfer (up to 700 µl) the mixture to a mini column and centrifuge at ≥10,000 x g for 30 sec.
Wash	Add 500 µl Buffer RBW to the mini column and centrifuge at ≥10,000 x g for 30 sec. Add 500 µl Buffer RNW to the mini column and centrifuge at ≥10,000 x g for 30 sec. Centrifuge at ≥10,000 x g for an additional 1 min.
Elution	Add ~50 µl Nuclease-free water to the center of the membrane in the mini column. Centrifuge at ≥10,000 x g for 1 min.

Brief Protocol



INDEX

Kit Contents

Cat. No.	315-150	
Components	**Quantity**	**Storage**
RiboEx™ LS	50 ml	2~8°C
Buffer RB1 (concentrate) *	15 ml	Room temperature (15~25°C)
Buffer RBW (concentrate) *	13 ml	
Buffer RNW (concentrate) * †	6 ml	
Nuclease-free water	15 ml	
EzPure™ Filter (with collection tube)	50	
Column Type W (mini) (with collection tube)	50	
1.5 ml microcentrifuge tube	50	
Protocol Handbook	1	

** Before first use, add absolute ethanol (ACS grade or better) into Buffer RB1, RBW and RNW as indicated on the bottle.*

† Contains sodium azide as a preservative

Materials Not Provided

Reagent : Absolute ethanol (ACS grade or better),
Cloroform or 1-bromo-3-chloropropane (BCP)

Disposable material : RNase-free pipette tips, Disposable gloves

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

Product Specifications

Hybrid-R™ Blood RNA	
Type	Spin
Maximum amount of starting samples	0.25 ml
Minimum amount of starting samples	0.1 ml
Maximum loading volume	700 μl
Minimum elution volume	30 μl
Maximum binding capacity	100 μg

Quality Control

All components of GeneAll® Hybrid-R™ Blood RNA are manufactured in strictly clean conditions, and their degree of cleanliness is monitored periodically.
To maintain consistency, a quality control process is carried out throughly from lot to lot and only the qualified kits are approved to be delivered.

Storage Conditions

All components of GeneAll® Hybrid-R™ Blood RNA, except RiboEx™ LS, should be stored at room temperature. RiboEx™ LS should be stored at 4°C for optimal performance.
GeneAll® Hybrid-R™ Blood RNA is guaranteed until the expiration date printed on the product box.

Safety Information

The buffers included in the GeneAll® Hybrid-R™ Blood RNA 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™ LS contains phenol which is poisonous and guanidine salt 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® Hybrid-R™ Blood RNA 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 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.

Product Description

Intended Use

Hybrid-R™ Blood RNA is suitable for RNA preparation from 0.1 ml to 0.25 ml mammalian whole blood. The typical yield is 3 μg per 0.25 ml whole blood. The purified RNA can be applicable for the isolation of Poly A^+ RNA, Northern blotting, dot blotting, in vitro translation, cloning, RT-PCR, RPA and other analytical procedures.

General Description

Hybrid-R™ Blood RNA is a complete kit with ready-to-use reagent for the isolation of total RNA from up to 0.25 ml whole blood sample.

This kit utilizes the lysis method of RiboEx™ LS which has a powerful ability of cell-lysis and the purification method based on glassfiber membrane technology. Fast and convenient procedure of Hybrid-R™ Blood RNA takes only 30 minutes for complete preparation of pure RNA.

Hybrid-R™ Blood RNA does not need the additional treatment of blood sample, and whole blood is lysed in RiboEx™ LS in just one step. Then addition of chloroform brings about a separation of the lysate into aqueous and organic phases. After phase-separating, DNA and protein remains in the interphase and the organic phase respectively but released RNA exists in the aqueous phase.

The aqueous phase is picked and applied to a EzPure™ Filter to eliminate small amount of contaminated DNA and other blood contaminants. The passed-through is mixed with Buffer RB1, RNA binding buffer, and then the mixture is applied to a mini column. After a series of washing with Buffer RBW and RNW, pure RNA can be eluted by Nuclease-free water.

Hybrid-R™ Blood RNA

PROTOCOL FOR RNA ISOLATION

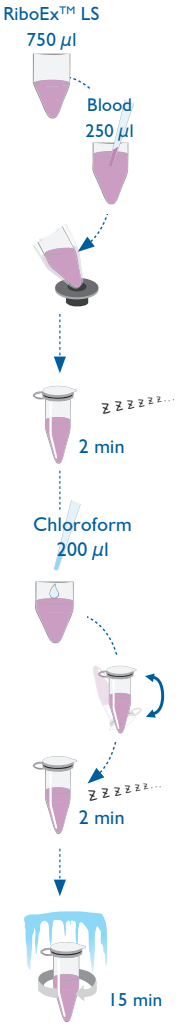
1. **Prepare 750 µl RiboEx™ LS in a 1.5 ml microcentrifuge tube (not provided).**

2. **Add 250 µl blood sample to the 1.5 ml microcentrifuge tube and vortex vigorously.**
   If sample volume is less than 100 µl, sample should be adjusted to 250 µl with PBS or Nuclease-free water.
   Be sure to confirm the applicable minimum volume, which is 100 µl.

3. **Incubate for 2 min at room temperature.**
   This step allows leukocytes to completely be collapsed.

4. **Add 0.2 ml chloroform. Shake vigorously for 15 sec and let it stand for 2 min at room temperature.**
   Alternatively, 0.1 ml 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**
   The mixture will be separated into three phases; a lower layer, an interphase, and a colorless upper aqueous layer. The upper aqueous volume is about 450 µl.
   Centrifugation at temperatures >8°C may cause some DNA to partition in the aqueous phase.



6. **Transfer the aqueous phase (approximately 450 µl) to a EzPure™ Filter (yellow).**
   Small amount of DNA and other blood contaminants are eliminated by EzPure™ Filter.

7. **Centrifuge at ≥10,000 x g for 30 sec at room temperature.**

8. **Add 2 volume (usually 900 µl) of Buffer RB1 to the collection tube including passed-through, and mix well by pipetting.**
   Do not centrifuge at this step.

9. **Transfer upto 700 µl of the mixture to a Column Type W (mini) (blue ring).**

10. **Centrifuge at ≥10,000 x g for 30 sec at room temperature.**
    Discard the passed-through and reinsert the mini column back into the same tube.

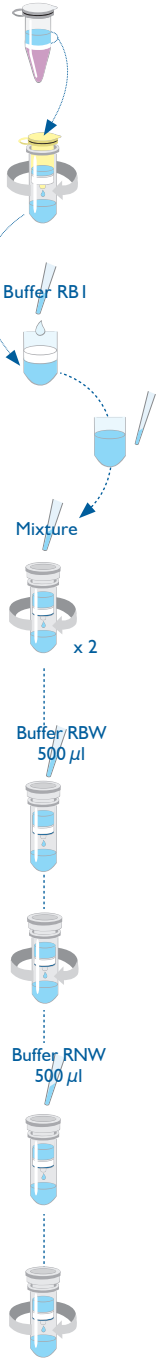
11. **Repeat step 9~10 using the remainder of the sample.**

12. **Add 500 µl Buffer RBW to the mini column.**

13. **Centrifuge at ≥10,000 x g for 30 sec at room temperature.**
    Discard the passed-through and reinsert the mini column back into the same tube.

14. **Add 500 µl Buffer RNW to the mini column.**

15. **Centrifuge at ≥10,000 x g for 30 sec at room temperature.**
    Discard the passed-through and reinsert the mini column back into the same tube.



16. Centrifuge at ≥10,000 x 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.

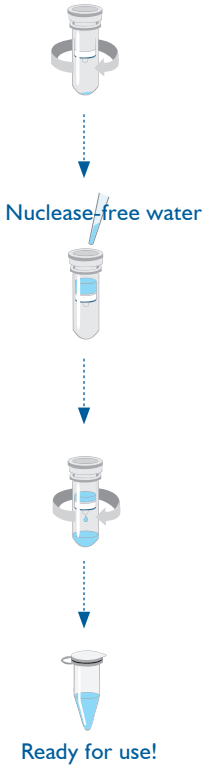
17. Add 50 μl Nuclease-free water to the center of the membrane in the mini column.

To increase the RNA concentration, reduce the elution volume at least 30 μl.

18. Centrifuge at ≥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.

The purified RNA is free of DNA and proteins, and A_{260}/A_{280} will be between 1.8 and 2.2.



Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low yield of RNA	**Poor quality of blood sample**	Too old or improperly stored sample often yield degraded RNA. Use fresh blood sample immediately. Repeated freezing and thawing the sample should be avoided.
	Sample not lysed completely	Vortex the sample vigorously. Be sure to incubate for 2 min at room temperature after lysis step.
	Some aqueous phase left	Perform second extraction with the remaining aqueous phase.
	Too much or less blood sample	It may cause an inefficient lysis effect. Use the appropriate sample volume from 100 µl to 250 µl.
	Incorrect elution conditions	Add Nuclease-free water to the center of the mini column membrane.
Degradation of RNA	**Sample manipulated too much before the addition of RiboEx™ LS**	Process the sample immediately after harvest.
	Too long storage of blood sample	Store blood sample at -70°C (not be recommended). As storing time goes on, RNA condition will be poorer.
	Reagent or disposable is not RNase-free	Make sure to use RNase-free products only.
Low A_{260}/A_{280} (<1.6)	**Aqueous phase was contaminated with the phenol phase**	Avoid carryover when transferring the aqueous phase to a EzPure™ Filter.
	Sample not completely lysed with RiboEx™ LS	Use 750 µl RiboEx™ LS for up to 250 µl blood sample. Be sure to incubate sample for 2 min at room temperature after lysis step.

Troubleshooting Guide

Facts	Possible Causes	Suggestions
Contamination of DNA	**The interphase was co-transferred by mistake**	Be sure not to transfer any of the interphase (containing DNA) to the aqueous phase.
	Insufficient RiboEx™ LS used or overused sample volume	Use the appropriate sample volume from 100 μl to 250 μl/750 μl RiboEx™ LS.
	Missed EzPure™ Filter step	Be sure to obey step 6 (page 11). This step eliminate the contaminated small amount of DNA.
	Temperature was too high during centrifugation	The phase separation should be performed at 4°C to allow optimal phase separation and removal of genomic DNA from the aqueous phase.
RNA does not perform well in downstream application	**Residual ethanol remains in eluate**	Centrifuge again to remove any residual ethanol included in Buffer RNW from mini column membrane (step 16 of page 12).

APPENDIX I. 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 not, 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 μg RNA/ml at a neutral pH. Therefore, the concentration of RNA was calculated by the formula shown below.

A_{260} x dilution factor x 40 = RNA μg/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 2. 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 10 X MOPS buffer, 18 ml of 37% formaldehyde, and 1 μl of a 10 mg/ml ethidium bromide (EtBr).
4. Mix well then pour the gel into the gel tray and cool to solidify it.
5. Transfer the solidified gel from tray to tank, and add enough 1 X MOPS running buffer to cover the gel.

Prepare the RNA sample

1. Make the mixture.
   ? μl RNA (up to 20 μg)
   2 μl 10 X MOPS electrophoresis buffer
   4 μl formaldehyde
   10 μl formamide
2. Incubate the mixture for 15 min at 65°C.
3. Chill the sample for 5 min in ice.
4. Add 2 μl of 10 X formaldehyde gel-loading dye to the mixture.
5. Load the mixture in a denaturing gel which is covered with a sufficient 1 X MOPS electrophoresis buffer.
6. Run the gel and confirm the RNA band on transilluminator.
   Occasionally, gel destaining may be needed to increase the visibility of the bands of RNA in dH_2O for several hours.

Composition of buffers

- 10 X MOPS buffer
  0.2 M MOPS
  20 mM sodium acetate
  10 mM EDTA
  pH to 7.0 with NaOH

- 10 X formaldehyde gel-loading dye
  50% glycerol
  10 mM EDTA
  0.25% (w/v) bromophenol blue
  0.25% (w/v) xylene cyanol FF

*** Caution**

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

Ordering Information

GeneAll® *Hybrid-Q™* *for rapid preparation of plasmid DNA*

Products	Scale	Size	Cat. No.	Type
Plasmid Rapidprep	mini	50	100-150	spin
		200	100-102	

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

Products	Scale	Size	Cat. No.	Type
Plasmid SV	mini	50	101-150	spin / vacuum
		200	101-102	
	Midi	26	101-226	spin / vacuum
		50	101-250	
		100	101-201	

GeneAll® *Exfection™* *for preparation of transfection-grade plasmid DNA*

Products	Scale	Size	Cat. No.	Type
Plasmid LE *(Low Endotoxin)*	mini	50	111-150	spin / vacuum
		200	111-102	
	Midi	26	111-226	spin / vacuum
		100	111-201	
Plasmid EF *(Endotoxin Free)*	Midi	20	121-220	spin
		100	121-201	

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

Products	Scale	Size	Cat. No.	Type
Gel SV	mini	50	102-150	spin / vacuum
		200	102-102	
PCR SV	mini	50	103-150	spin / vacuum
		200	103-102	
CleanUp SV	mini	50	113-150	spin / vacuum
		200	113-102	
Combo GP	mini	50	112-150	spin / vacuum
		200	112-102	

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

Products	Scale	Size	Cat. No.	Type
Tissue SV	mini	100	104-101	spin / vacuum
		250	104-152	
	Midi	26	104-226	spin / vacuum
		100	104-201	
	MAXI	10	104-310	spin / vacuum
		26	104-326	
Tissue Plus SV	mini	100	109-101	spin / vacuum
		250	109-152	
	Midi	26	109-226	spin / vacuum
		100	109-201	
	MAXI	10	109-310	spin / vacuum
		26	109-326	

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

Products	Scale	Size	Cat. No.	Type
Blood SV	mini	100	105-101	spin / vacuum
		250	105-152	
	Midi	26	105-226	spin / vacuum
		100	105-201	
	MAXI	10	105-310	spin / vacuum
		26	105-326	
Cell SV	mini	100	106-101	spin / vacuum
		250	106-152	
	MAXI	10	106-310	spin / vacuum
		26	106-326	
Clinic SV	mini	100	108-101	spin / vacuum
		250	108-152	
	Midi	26	108-226	spin / vacuum
		100	108-201	
	MAXI	10	108-310	spin / vacuum
		26	108-326	
Genomic DNA micro		50	118-050	spin
Plant SV	mini	100	117-101	spin / vacuum
		250	117-152	
	Midi	26	117-226	spin / vacuum
		100	117-201	
	MAXI	10	117-310	spin / vacuum
		26	117-326	
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*

Products	Scale	Size	Cat. No.	Type
GenEx™ Blood	Sx	100	220-101	solution
		500	220-105	
	Lx	100	220-301	solution
GenEx™ Cell	Sx	100	221-101	solution
		500	221-105	
	Lx	100	221-301	solution
GenEx™ Tissue	Sx	100	222-101	solution
		500	222-105	
	Lx	100	222-301	solution

Products	Scale	Size	Cat. No.	Type
GeneAll® *GenEx™* *for isolation of total DNA without spin column*				
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 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	260-031	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 preperation 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
GeneAll® *AmpONE™* *for PCR amplification*				
Taq DNA polymerase		250 U	501-025	(2.5 U/μl)
		500 U	501-050	
		1,000 U	501-100	
Taq Premix	20 μl x 96 tubes		526-200	solution
	50 μl x 96 tubes		526-500	
GeneAll® *AmpMaster™* *for PCR amplification*				
Taq Master mix	0.5 ml x 2 tubes		541-010	solution
	0.5 ml x 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

GeneAll® Protein series

ProtinEx™ 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

GeneAll® GENTi™ 32 ADVANCED *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

Symbol

Symbol	Used for	Symbol	Used for
REF	Catalog number	IVD	In vitro diagnostic medical device
LOT	Batch number	EC REP	European Authorized Representative
	Use by		Consult instruction for use
	Manufacturer information		Do not reuse
	Production date		Temperature limitation
	Important note	CONC	Contains the concentrated solution. Additional material must be added before use
CE	CE-Mark		Caution
? EtOH ____	Write down the current date after adding ethanol to the bottle	EtOH ?	Mark up after adding ethanol

NOTE

NOTE

GeneAll

GENEALL BIOTECHNOLOGY CO., LTD

GENEALL BIOTECHNOLOGY CO., LTD
GeneAll Bldg., 303-7, Dongnamro, Songpa-gu, Seoul, Korea 05729
E-mail : sales@geneall.com
Tel. 82-2-407-0096 Fax. 82-2-407-0779
www.geneall.com

Manufacturer site

A-1201~A-1204, Hanam Techno Valley U1 Center,
947, Hanam-daero, Hanam-si, Gyeonggi-do, 12982, Korea

EC REP

MT Promedt Consulting GmbH
Ernst-Heckel-Straße 7, 66386 St. Ingbert,
Germany
Tel. 49 6894 581020
Fax. 49 6894 581021

2023.07