

Ver 1.0

Cat.No. 315-150

# Hybrid-R™ Blood RNA

BLOOD TOTAL RNA PURIFICATION HANDBOOK



## Customer & Technical Support

Do not hesitate to ask us any question.

We thank you for any comment or advice.

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This protocol handbook is included in :

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

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## Homogenization

Lyse ~ 250 ul whole blood / 750 ul RiboEx™ LS.

Incubate the lysate for 2 minutes at RT

## Phase separation

Add 200 ul chloroform

Incubate the mixture for 2 minutes at RT

Centrifuge at 12,000 x g for 15 minutes at 4°C.

## EzPure™ filter step

Transfer the aqueous phase to a EzPure™ filter and centrifuge at  $\geq 10,000 \times g$  for 30 seconds.

## RNA bind

Add 2 volume of buffer RBI to the collection tube including passed-through and mix thoroughly by pipetting.

Transfer (up to 700 ul) the mixture to a mini spin column and centrifuge at  $\geq 10,000 \times g$  for 30 seconds.

## RNA wash

Add 500 ul of buffer RBW to the mini spin column and centrifuge at  $\geq 10,000 \times g$  for 30 seconds.

Add 500 ul of buffer RNW to the mini spin column and centrifuge at  $\geq 10,000 \times g$  for 30 seconds.

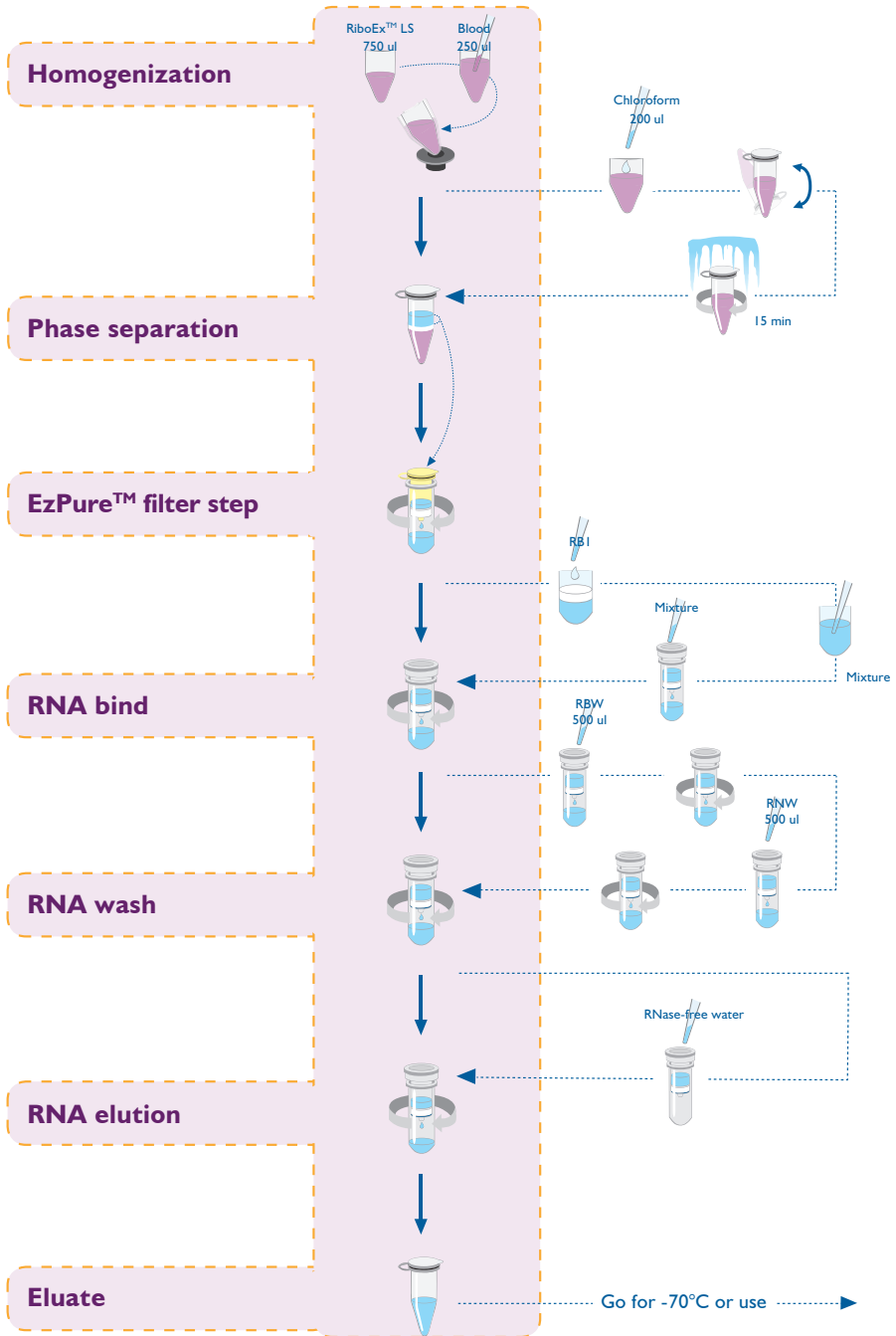
Centrifuge at  $\geq 10,000 \times g$  for an additional 1 minute.

## RNA elution

Add ~50 ul of free-water to the center of the membrane in the mini spin column.

Centrifuge at  $\geq 10,000 \times g$  for 1 minute.

# Brief protocol



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# KIT CONTENTS

Components	Quantity	Storage
RiboEx™ LS	50 ml	4°C
Buffer RB1	60 ml	Room temperature
Buffer RBW	30 ml	
Buffer RNW	30 ml	
RNase-free water	15 ml	
GeneAll® EzPure™ filter (yellow) (with collection tube)	50	
GeneAll® Column type W (blue ring) (with collection tube)	50	
1.5 ml collection tube	50	

## Materials Not Provided

### Reagent

- Chloroform or 1-bromo-3-chloropropane(BCP)

### Disposable material

- RNase-free pipet tips
- Disposable gloves

### Equipment

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

## Quality Control

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Hybrid-R™ Blood RNA 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

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Hybrid-R™ Blood RNA kit except RiboEx™ LS solution should be stored at room temperature.

RiboEx™ LS solution should be stored at 4°C for optimal performance.

All components are stable for 1 year.

## Precautions

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RiboEx™ LS contains phenol which is poisonous and guanidine salt which is an irritant. When working with Hybrid-R™ Blood RNA, use gloves and eye protector to avoid contact with skin or clothing and inhalation of vapor. In case of contact, wash immediately with plenty of water and seek medical advice.

## Product Disclaimer

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Hybrid-R™ Blood RNA kit is for research use only, not for use in diagnostic procedure.

## Product Specifications

Specification	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 ul
Minimum elution volume	30 ul
Maximum binding capacity	100 ug

## 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.



## Product 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.

Whole blood sample is homogenized and lysed in RiboEx™ LS, a monophasic solution containing phenol and guanidium salt, which rapidly lyse cells and inactivates nucleases. In conventional methods, the erythrocytes of mammalian blood which does not contain nuclei (and therefore, RNA either) should be removed by pre-treatment such as osmotic lysis for the separation of leukocytes from whole blood. This additional treatments increase the experiment time and the possibility of RNA-breakage, followed by decline of RNA-quality.

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 RBI, RNA binding buffer, and then the mixture is applied to a mini spin column. After a series of washing with buffer RBW and RNW, pure RNA can be eluted by RNase-free water.

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 ug per 0.25 ml whole blood. The purified RNA can be applicable for the isolation of Poly A<sup>+</sup> RNA, Northern blotting, dot blotting, in vitro translation, cloning, RT-PCR, RPA and other analytical procedures.

# Hybrid-R™ Blood RNA

## PROTOCOL FOR RNA isolation

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

- 2. Add 250 ul blood sample to the 1.5 ml microcentrifuge tube and vortex vigorously.**

If sample volume is 100 ul, sample should be added to 250 ul with PBS or RNase-free water.

Be sure to confirm the applicable minimum volume, which is 100 ul.

- 3. Incubate 2 min at room temperature.**

This step allows leukocytes to completely be collapsed.

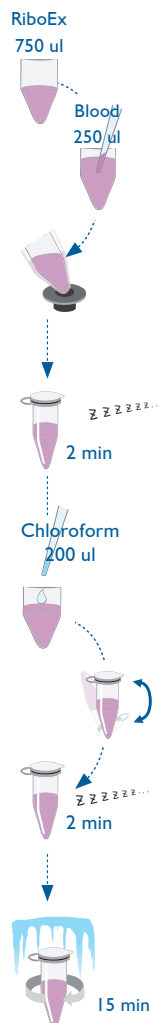
- 4. Add 0.2 ml of chloroform. Shake vigorously for 15 seconds and let it stand for 2 minutes at room temperature.**

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 minutes 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 ul.

Centrifugation at temperatures  $>8^{\circ}\text{C}$  may cause some DNA to partition in the aqueous phase.



**6. Transfer the aqueous phase (approximately 450 ul) to a EzPure™ filter (yellow).**

Small amount of DNA and other blood contaminants are eliminated by EzPure™ filter.

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

**8. Add 2 volume (usually 900 ul) of buffer RBI to the collection tube including passed-through, and mix well by pipetting.**

Do not centrifuge at this step.

**9. Transfer upto 700 ul of the mixture to a mini spin column (type W, blue ring).**

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

Discard the passed-through and reinsert the mini spin column back into the same tube.

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

**12. Add 500 ul of buffer RBW to the mini spin column.**

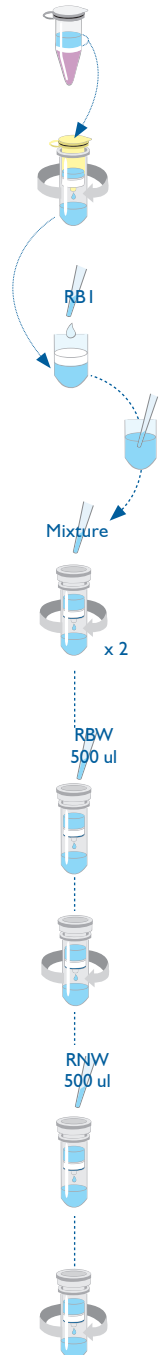
**13. Centrifuge at  $\geq 10,000 \times g$  for 30 seconds at room temperature.**

Discard the passed-through and reinsert the mini spin column back into the same tube.

**14. Add 500 ul of buffer RNW to the mini spin column.**

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

Discard the passed-through and reinsert the mini spin column back into the same tube.



**16. Centrifuge at  $\geq 10,000 \times g$  for an additional 1 minute at room temperature to remove residual wash buffer. Transfer the mini spin 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.

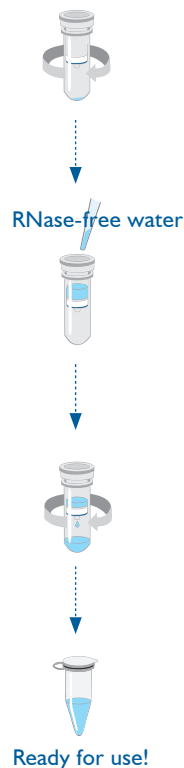
**17. Add 50  $\mu$ l of RNase-free water to the center of the membrane in the mini spin column.**

To increase the RNA concentration, reduce the elution volume at least 30  $\mu$ l.

**18. Centrifuge at  $\geq 10,000 \times g$  for 1 minute 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
<b>Low yield of RNA</b>	<b>Poor quality of blood sample</b>	Process the fresh blood sample immediately .
	<b>Sample not lysed completely</b>	Vortex the sample vigorously. Be sure to incubate for 2 minutes at room temperature after lysis step.
	<b>Some aqueous phase left</b>	Perform second extraction with the remaining aqueous phase.
	<b>Too much or less blood sample</b>	It may cause an inefficient lysis effect. Use the appropriate sample volume from 100 ul to 250 ul.
	<b>Incorrect elution conditions</b>	Add RNase-free water to the center of the mini spin column membrane.
<b>Degradation of RNA</b>	<b>Sample manipulated too much before the addition of RiboEx™ LS</b>	Process the sample immediately after harvest.
	<b>Too long storage of blood sample</b>	Store blood sample at -70°C (not be recommended). As storing time goes on, RNA condition will be poorer.
	<b>Reagent or disposable is not RNase-free</b>	Make sure to use RNase free products only.
<b>Low A<sub>260/280</sub> (&lt;1.6)</b>	<b>Aqueous phase was contaminated with the phenol phase</b>	Avoid carryover when transferring the aqueous phase to a EzPure™ filter.
	<b>Sample not completely lysed with RiboEx™ LS</b>	Use 750 ul RiboEx™ LS for up to 250 ul blood sample.  Be sure to incubate sample for 2 minutes at room temperature after lysis step.

## Troubleshooting Guide

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

# 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 ug RNA / ml at a neutral pH. Therefore, the concentration of RNA was calculated by the formula shown below.

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

## 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 1g 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 ul 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.
  - ? ul RNA (up to 20 ug)
  - 2 ul 10 X MOPS electrophoresis buffer
  - 4 ul formaldehyde
  - 10 ul formamide
2. Incubate the mixture for 15 minutes at 65°C.
3. Chill the sample for 5 minutes in ice.
4. Add 2 ul 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<sub>2</sub>O 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

Products	Size	Type	Cat. No.
<b>GeneAll® Hybrid-Q™</b> for rapid preparation of plasmid DNA			
Plasmid Rapidprep	50	mini / spin	100-150
	200		100-102
<b>GeneAll® Exprep™</b> for preparation of plasmid DNA			
Plasmid SV mini	50	spin / vacuum	101-150
	200		101-102
	1,000		101-111
Plasmid SV Midi**	26	spin / vacuum	101-226
	50		101-250
	100		101-201
Plasmid SV Quick	50	mini / spin	101-050
	200		101-002
	1,000		101-011
<b>GeneAll® Exfection™</b> for preparation of highly pure plasmid DNA			
Plasmid LE mini (Low Endotoxin)	50	spin / vacuum	111-150
	200		111-102
Plasmid LE Midi* (Low Endotoxin)	26	spin / vacuum	111-226
	100		111-201
Plasmid EF Midi* (Endotoxin Free)	20	spin	121-220
	100		121-201
<b>GeneAll® Expin™</b> for purification of fragment DNA			
Gel SV	50	mini / spin / vacuum	102-150
	200		102-102
PCR SV	50	mini / spin / vacuum	103-150
	200		103-102
CleanUp SV	50	mini / spin / vacuum	113-150
	200		113-102
Combo GP	50	mini / spin / vacuum	112-150
	200		112-102
<b>GeneAll® Exgene™</b> for isolation of total DNA			
Tissue SV mini (plus!)*	100	spin / vacuum	104(9)-101
	250		104(9)-152
Tissue SV Midi (plus!)**	26	spin / vacuum	104(9)-226
	100		104(9)-201
Tissue SV MAXI (plus!)**	10	spin / vacuum	104(9)-310
	26		104(9)-326
Blood SV mini	100	spin / vacuum	105-101
	250		105-152
Blood SV Midi**	26	spin / vacuum	105-226
	100		105-201
Blood SV MAXI**	10	spin / vacuum	105-310
	26		105-326

Products	Size	Type	Cat. No.
<b>GeneAll® Exgene™</b> for isolation of total DNA			
Cell SV mini	100	spin / vacuum	106-101
	250		106-152
Cell SV MAXI**	10	spin / vacuum	106-310
	26		106-326
Clinic SV mini	100	spin / vacuum	108-101
	250		108-152
Clinic SV Midi	26	spin / vacuum	108-226
	100		108-201
Clinic SV MAXI**	10	spin / vacuum	108-310
	26		108-326
Genomic DNA micro	50	spin	118-050
Plant SV mini	100	spin / vacuum	117-101
	250		117-152
Plant SV Midi**	26	spin / vacuum	117-226
	100		117-201
Plant SV MAXI**	10	spin / vacuum	117-310
	26		117-326
GMO SV mini	50	spin / vacuum	107-150
	200		107-102
<b>GeneAll® GenEx™</b> for isolation of total DNA			
GenEx™ B	100 <sup>†</sup>	mini / solution	220-101
	500 <sup>†</sup>		220-105
	100 <sup>††</sup>		220-301
GenEx™ C	100 <sup>†</sup>	mini / solution	221-101
	500 <sup>†</sup>		221-105
	100 <sup>††</sup>		221-301
GenEx™ T	100 <sup>†</sup>	mini / solution	222-101
	500 <sup>†</sup>		222-105
	100 <sup>††</sup>		222-301
<b>GeneAll® DirEx™</b> Single tube DNA extraction buffer for PCR			
DirEx™	50	solution	250-050

\* GeneAll® Tissue SV mini, Midi, and MAXI plus kit provide the additional methods for the purification from animal whole blood.

\*\* 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 ul whole blood, 2 x 10<sup>8</sup> cells or 10 mg animal tissue.

†† On the basis of DNA purification from 10 ml whole blood. 1 x 10<sup>8</sup> cells or 100 mg animal tissue.

# Ordering Information

Products	Size	Type	Cat. No.
<b>GeneAll® RiboEx™</b> for preparation of total RNA			
RiboEx™	100	solution	301-001
	200		301-002
Hybrid-R™	100	spin	305-101
Hybrid-R™ Blood RNA	50	spin	315-150
Hybrid-R™ miRNA	50	spin	325-150
RiboEx™ LS	100	solution	302-001
	200		302-002
Riboclear™	50	spin	303-150
Ribospin™	50	spin	304-150
Ribospin™ vRD	50	spin	302-150
Allspin™	50	spin	306-150
<b>GeneAll® AmpONE™</b> for PCR amplification			
Taq DNA polymerase	250 U	(2.5 U/μL)	501-025
	500 U		501-050
	1,000 U		501-100
α-Taq DNA polymerase	250 U	(2.5 U/μL)	502-025
	500 U		502-050
	1,000 U		502-100
Pfu DNA polymerase	250 U	(2.5 U/μL)	503-025
	500 U		503-050
	1,000 U		503-100
Hotstart Taq DNA polymerase	250 U	(2.5 U/μL)	531-025
	500 U		531-050
	1,000 U		531-100
Clean Taq DNA polymerase	250 U	(2.5 U/μL)	551-025
	500 U		551-050
	1,000 U		551-100
Clean α-Taq DNA polymerase	250 U	(2.5 U/μL)	552-025
	500 U		552-050
	1,000 U		552-100
Taq Master mix	2x	0.5 ml x 2 tubes	511-010
	2x	0.5 ml x 10 tubes	511-050
α-Taq Master mix	2x	0.5 ml x 2 tubes	512-010
	2x	0.5 ml x 10 tubes	512-050

\* GeneAll® Tissue SV mini, Midi, and MAXI plus! kit provide the additional methods for the purification from animal whole blood.

\*\* 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 ul whole blood, 2 x 10<sup>6</sup> cells or 10 mg animal tissue.

†† On the basis of DNA purification from 10 ml whole blood. 1 x 10<sup>6</sup> cells or 100 mg animal tissue.

Products	Size	Type	Cat. No.
<b>GeneAll® AmpONE™</b> for PCR amplification			
Taq Premix	20 $\mu\ell$ 50 $\mu\ell$	96 tubes	521-200 521-500
$\alpha$ -Taq Premix	20 $\mu\ell$ 50 $\mu\ell$	96 tubes	522-200 522-500
Taq Premix (w/o dye)	20 $\mu\ell$	96 tubes	524-200
$\alpha$ -Taq Premix (w/o dye)	20 $\mu\ell$	96 tubes	525-200
dNTP mix	500 $\mu\ell$	2.5 mM each	509-020
dNTP set (set of dATP, dCTP, dGTP and dTTP)	1 ml x 4 tubes	100 mM	509-040

\* Each dNTP is available







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