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 :

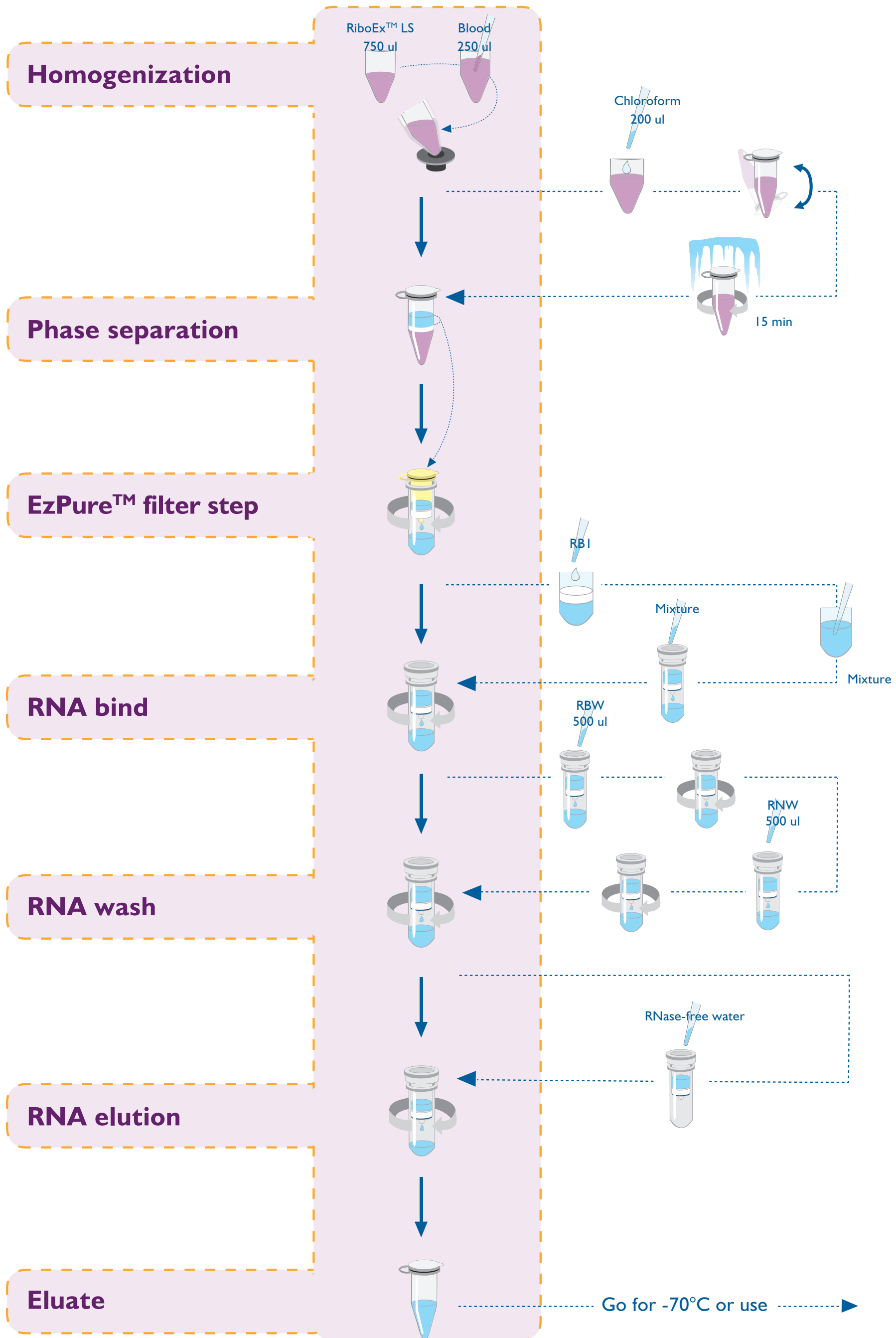
GeneAll® Hybrid-R™ Blood RNA (315-150)

Visit www.geneall.com or www.geneall.co.kr for FAQ, QnA and more information.

Brief protocol

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 ≥ 10,000 x g for 30 seconds.
RNA bind	Add 2 volume of buffer RB1 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 ≥ 10,000 x g for 30 seconds.
RNA wash	Add 500 ul of buffer RBW to the mini spin column and centrifuge at ≥ 10,000 x g for 30 seconds. Add 500 ul of buffer RNW to the mini spin column and centrifuge at ≥ 10,000 x g for 30 seconds. Centrifuge at ≥ 10,000 x 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 ≥ 10,000 x 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

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

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

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

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.

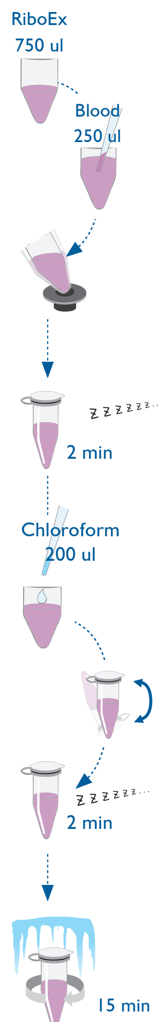
Hybrid-R™ Blood RNA does not need the additional treatment of blood sample, and whole blood is lysed in RiboEx™ LS in just one step. Then addition of chloroform brings about a separation of the lysate into aqueous and organic phases. After phase-separating, DNA and protein remains in the interphase and 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 RB1, 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^+ 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 adjusted 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°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 ≥ 10,000 x g for 30 seconds at room temperature.**

8. **Add 2 volume (usually 900 ul) 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 ul of the mixture to a mini spin column (type W, blue ring).**

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

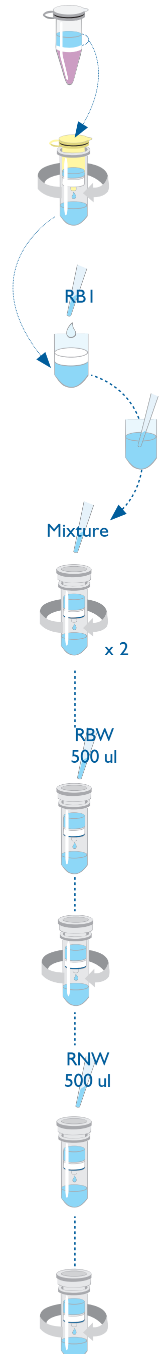
11. **Repeat step 9 ~ 10 using the remainder of the sample.**

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

13. **Centrifuge at ≥ 10,000 x 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 ≥ 10,000 x 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 ≥ 10,000 x 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.

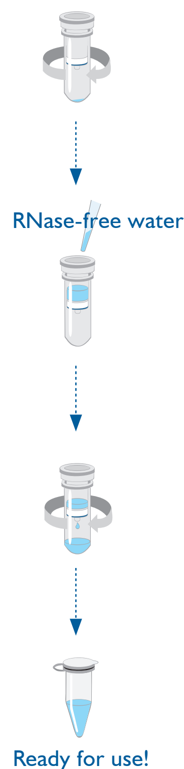
17. Add 50 ul of RNase-free water to the center of the membrane in the mini spin column.

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

18. Centrifuge at ≥ 10,000 x 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
Low yield of RNA	**Poor quality of blood sample**	Process the fresh blood sample immediately .
	Sample not lysed completely	Vortex the sample vigorously. Be sure to incubate for 2 minutes 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 ul to 250 ul.
	Incorrect elution conditions	Add RNase-free water to the center of the mini spin 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 $A_{260/280}$ (<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 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
Contamination of DNA	**The interphase was co-transfered 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 ul to 250 ul / 750 ul RiboEx™ LS.
	Missed EzPure™ filter step	Be sure to obey step 6. 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 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_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

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

Products	Size	Type	Cat. No.
GeneAll® *Exgene™* *for isolation of total DNA*			
Cell SV mini	100 250	spin / vacuum	106-101 106-152
Cell SV MAXI**	10 26	spin / vacuum	106-310 106-326
Clinic SV mini	100 250	spin / vacuum	108-101 108-152
Clinic SV Midi	26 100	spin / vacuum	108-226 108-201
Clinic SV MAXI**	10 26	spin / vacuum	108-310 108-326
Genomic DNA micro	50	spin	118-050
Plant SV mini	100 250	spin / vacuum	117-101 117-152
Plant SV Midi**	26 100	spin / vacuum	117-226 117-201
Plant SV MAXI**	10 26	spin / vacuum	117-310 117-326
GMO SV mini	50 200	spin / vacuum	107-150 107-102
GeneAll® *GenEx™* *for isolation of total DNA*			
GenEx™ B	100† 500† 100††	mini / solution mini / solution MAXI / solution	220-101 220-105 220-301
GenEx™ C	100† 500† 100††	mini / solution mini / solution MAXI / solution	221-101 221-105 221-301
GenEx™ T	100† 500† 100††	mini / solution mini / solution MAXI / solution	222-101 222-105 222-301
GeneAll® *DirEx™* *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 10^6 cells or 10 mg animal tissue.
†† On the basis of DNA purification from 10 ml whole blood. 1 x 10^8 cells or 100 mg animal tissue.

Ordering Information

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

** 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 10^6 cells or 10 mg animal tissue.
†† On the basis of DNA purification from 10 ml whole blood. 1 x 10^8 cells or 100 mg animal tissue.

Products	Size	Type	Cat. No.
GeneAll® *AmpONE™* *for PCR amplification*			
Taq Premix	20 μℓ 50 μℓ	96 tubes	521-200 521-500
α-Taq Premix	20 μℓ 50 μℓ	96 tubes	522-200 522-500
Taq Premix (w/o dye)	20 μℓ	96 tubes	524-200
α-Taq Premix (w/o dye)	20 μℓ	96 tubes	525-200
dNTP mix	500 μℓ	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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