Ver 1.0

Cat.No. 307-150

Ribospin™ Plant

PLANT TOTAL RNA PURIFICATION HANDBOOK

GeneAll

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® Ribospin™ Plant (307-150)

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

Brief protocol

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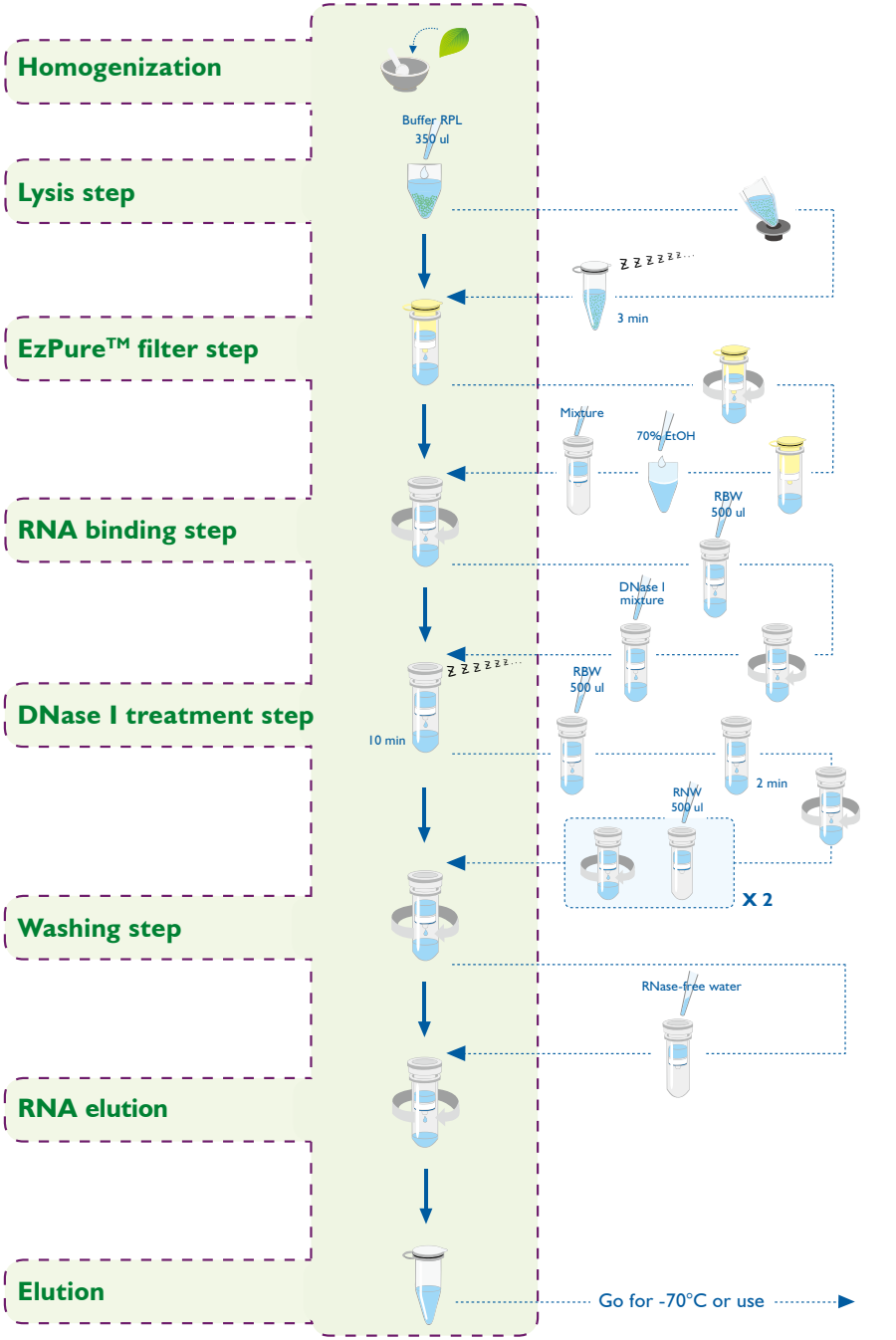
Homogenization	Homogenize ~100 mg / prep plant samples in liquid nitrogen. Transfer the powder into a 1.5ml microcentrifuge tube.
Lysis step	Add 350 ul of buffer RPL. Incubate the mixture for 3 min at R.T.
EzPure™ filter step	Transfer the lysate to a EzPure™ filter and centrifuge at ≥10,000 x g for 30 sec. Transfer the supernatant into a 1.5ml microcentrifuge tube
RNA binding step	Add 1 volume of 70% ethanol to the supernatant and mix well. Apply the mixture into a mini spin column and centrifuge at ≥10,000 x g for 30 sec.
DNase I treatment step	Add 500 ul of buffer RBW to the mini spin column and centrifuge at ≥10,000 x g for 30 sec. Apply the DNase I mixture into a mini spin column. Incubate the mixture for 10 min at R.T.
Washing step	Add 500 ul of buffer RBW to the mini spin column and incubate for 2 min and centrifuge at ≥10,000 x g for 30 sec. Add 500 ul of buffer RNW to the mini spin column and centrifuge at ≥10,000 x g for 30 sec. (twice) Centrifuge at ≥10,000 x g for an additional 1 min.
RNA elution	Add ~50 ul of RNase free water to the center of the membrane. Centrifuge at ≥10,000 x g for 1 min.

Brief protocol

GeneAll

GENEALL BIOTECHNOLOGY CO., LTD



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KIT CONTENTS

Components	Quantity	Storage
Buffer RPL	25 ml	Room temperature
Buffer REL	25 ml	
Buffer RBW	60 ml	
Buffer RNW	60 ml	
RNase-free water	15 ml	
Buffer DRB	5 ml	
GeneAll® EzPure™ filter (yellow) (with collection tube)	50	
GeneAll® Column type W (blue ring) (with collection tube)	50	
1.5 ml microcentrifuge tube	100	
DNase I	120 ul	- 20°C

Materials Not Provided

Reagent

- 70% ethanol, ACS grade or better

Disposable material

- RNase-free pipet tips
- Disposable gloves

Equipment

- Equipment for disrupting plant tissue
- Microcentrifuge

Quality Control

Ribospin™ Plant kit 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

Ribospin™ Plant kit, except DNase I, should be stored at room temperature (15 ~ 25°C). DNase I should be stored at -20°C.
All components are stable for 1 year.

Precautions

The buffers included in Ribospin™ Plant kit 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 RPL, REL, and RBW contain 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.

Product Disclaimer

Ribospin™ Plant kit is for research use only, This kit is not to be used for any other clinical test such as diagnostic, prognostic, therapeutic, etc.

Product Specifications

Specification	Ribospin™ Plant
Type	Spin
Maximum amount of starting samples	~ 100 mg plant tissue
Maximum loading volume of spin column	~ 700 ul
Minimum elution volume	30 ul
Maximum binding capacity	~ 100 ug

Typical yields

	Sample type	Amount of starting material	Typical yield
Leaf	*Pinus densiflora* (Pine)	100 mg	2.7 ug
	Cucumis sativus L. (Cucumber)	100 mg	50 ug
	Zea mays (Corn)	100 mg	11 ug
	Capsicum annuum (Red pepper)	100 mg	22 ug
	Lycopersicum esculentum (Tomato)	50 mg	13 ug
	Lactuca sativa (Lettuce)	100 mg	29 ug
	Citrus grandis Osbek (Satsuma)	100 mg	4.6 ug
	Diospyros kaki (Persimmon)	100 mg	16 ug
	Crassula ovata (Crassula)	100 mg	3 ug
	Nicotiana tabacum (Tabacco)	50 mg	13 ug
Root	*Allium cepa* (Onion)	100 mg	8 ug
	Plantago asiatica (Plantain)	50 mg	2.5 ug
	Nicotiana tabacum (Tabacco)	50 mg	5.3 ug
Fruit	*Citrus grandis Osbek* (Satsuma)	50 mg	1.1 ug
Germ bud	*Allium cepa* (Onion)	100 mg	9 ug

Product Description

Ribospin™ Plant kit is specially designed for purification of total RNA from various plant tissues such as leaves, stems, roots and picky plant samples. This kit provides the optimized buffer and spin column, which is effective at removing polysaccharides and polyphenolic compounds and isolating intact plant RNA. All components of Ribospin™ Plant are ready for use, so any further preparation for experiment is not required.

The procedure of Ribospin™ Plant kit begins with the disruption of sample in liquid nitrogen using mortar and pestle. The disrupted sample can be lysed in buffer RPL or REL. In most case, buffer RPL is the best buffer for lysis. However in some plant samples, solidification of lysate can be occurred with buffer RPL due to endosperm of seed or peculiar metabolites, and this can be avoided by using buffer REL as alternative for buffer RPL.

Most impurities except RNA in the lysate are eliminated by filtration through EzPure™ filter, and then the passed-through lysate is mixed with ethanol to adjust binding condition. Total RNA including a little impurity is bound to the membrane of spin column type W while the mixture is passing through. Survived genomic DNA can be exterminated by on-column DNase I treatment at this step. After a series of washing step using buffer RBW and RNW, plant total RNA is eluted by RNase-free water.

Whole procedure of Ribospin™ Plant takes only 25 minutes. The purified RNA is suitable for cDNA synthesis, RT-PCR, Northern blotting, and other analytical procedure.

PROTOCOL FOR

Ribospin™ Plant

Before starting

Prepare DNase I reaction mixture just before step 12.
Prepare aliquot DNase I and thaw on ice.
Mix 2 ul DNase I with 70 ul Buffer DRB.

1. **Prepare plant tissue sample upto 100 mg, then grind the sample to a fine powder using a mortar and pestle with liquid nitrogen and transfer the grinded sample into a 1.5 ml microcentrifuge tube (not provided).**

2. **Add 350 ul of buffer RPL to the 1.5 ml microcentrifuge tube and vortex vigorously.**
   In case of solidification of the lysate in buffer RPL, use buffer REL instead of buffer RPL.

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

4. **Transfer the lysate to a EzPure™ filter.**
   Through this step, large cell debris and most of genomic DNAs are filtered on the EzPure™ filter and small pellet of cell debris will be formed at the bottom of the collection tube.

5. **Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.**

6. **Transfer the supernatant to a new 1.5 ml microcentrifuge tube (provided).**
   Be careful not to disturb the pellet at the bottom of the collection tube.

7. **Add 1 volume (usually 350 ul) of 70% EtOH to the tube containing supernatant, and mix well by pipetting or inverting.**
   Do not centrifuge at this step.

8. Apply the mixture to a mini spin column (type W, blue ring).

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

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

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

12. Apply 70 ul of DNase I reaction mixture to the center of the mini spin column.
Incubate at the room temperature for 10 minutes.
To make DNase I reaction mixture, mix 2 ul DNase I with 70 ul Buffer DRB. DNase I is sensitive to physical damage and thus do not mix vigorously. If you want to DNase I treatment in RNA eluate, skip step 12 and 13 and refer to Appendix I "DNase I treatment in eluate".

13. Add 500 ul of buffer RBW to the mini spin column and stand for 2 minutes.
Buffer RBW inactivates DNase I and wash out the components of DNase I reaction buffer.

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

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

16. 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.

17. Repeat step 15 ~ 16.

18. 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.

19. 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 to 30 ul.

20. 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	**Sample not disrupted completely.**	Insufficient disruption can lead to decrease the yield of total RNA. Confirm the completley disrupted sample in liquid nitrogen and transfer the disrupted sample in a 1.5 ml tube.
	Too much starting sample	Overloading can decrease the yield of total RNA. Reduce the amount of starting sample.
	Poor quality of starting material	Process the sample immediately after harvest. To process later, freeze the sample rapidly in liquid nitrogen.
	Too low RNA mass in samples	Especially, some plant samples have low RNA content. To increase the RNA concentration, reduce the elution volume up to 30 ul or increase the amount of starting sample up to 100 mg per prep.
RNA degradation	**Sample manipulated too much before process**	Process the sample immediately after harvest. To process later, freeze the sample rapidly in liquid nitrogen.
	Improper storage of RNA	Store isolated RNA at -70°C, Do not store at -20°C.
	Reagent or disposable is not RNase-free	Make sure to use RNase-free products only.
EzPure™ filter clogging	**Sample not disrupted completely**	Insufficient disruption can clog the EzPure™ filter and to decrease the yield of total RNA. Confirm the complet disruption of the sample in liquid nitrogen.

Troubleshooting Guide

Facts	Possible Causes	Suggestions
DNA contamination of RNA eluate	**Too much starting sample**	Too much starting sample may leave lots of DNA fragments on the membrane over the activity of DNase I. Reduce starting sample used.
	Sample has high DNA mass	Some plant samples have high DNA content. In this case, some DNA can be eluted at RNA elution step. Reducing the amount of sample can reduce the genomic DNA contamination or Refer to the appendix I 'DNase I treatment in eluate'.
	DNase not active	For prolonged activity, aliquot the DNase I into small portion. Do not freezing and thawing the aliquots several times.
	Incorrect DNase I reaction treatment	Add DNase I reaction mixture to the center of the mini spin column membrane.
Eluate does not perform well in downstream application	**Residual ethanol remains in eluate**	To remove any residual ethanol included in buffer RNW from mini spin column membrane, centrifuge again for complete removal of ethanol.
	Buffer RBW and RNW used in wrong order	Ensure that buffer RBW and RNW are used in correct order. If used in the wrong order, wash the spin column with buffer RNW finally.

APPENDIX I. DNase I treatment in eluate

Appendix I describe how to use the DNase I (included in this kit) to eliminate contaminating DNA in RNA eluate. For samples containing high DNA contents, this method is strongly recommended. This procedure is more efficient than on-column DNase I treatment.

Protocol

1. Prepare the mixture as below in a microcentrifuge tube.
50 ul RNA eluate
5 ul Buffer DRB
1 ul DNase I

2. Incubate the mixture for 10 minutes at room temperature.

3. Re-elution of RNA.
Follow 3-1 or 3-2

DNase I treated RNA can be applied to RNA clean up kit (Riboclear™ cat no. 303-150). We strongly recommend using Riboclear™ kit for RNA clean up. Because ethanol precipitation and heat inactivation, usually used for DNase I inactivation, can damage the RNA.

3-1 Follow Riboclear™ protocol

3-2 Heat inactivation
1. Add 1 ul of 0.5M EDTA per 100 ul eluate.
2. Heat inactivate at 75°C for 10 minutes.

APPENDIX 2. 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} X dilution factor X 40 = 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 3. 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

Ordering Information

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.

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
Ribospin™ Plant	50	spin	307-150
Allspin™	50	spin	306-150
GeneAll® *AmpONE™* *for PCR amplification*			
Taq DNA polymerase	250 U 500 U 1,000 U	(2.5 U/㎕)	501-025 501-050 501-100
α-Taq DNA polymerase	250 U 500 U 1,000 U	(2.5 U/㎕)	502-025 502-050 502-100
Pfu DNA polymerase	250 U 500 U 1,000 U	(2.5 U/㎕)	503-025 503-050 503-100
Hotstart Taq DNA polymerase	250 U 500 U 1,000 U	(2.5 U/㎕)	531-025 531-050 531-100
Clean Taq DNA polymerase	250 U 500 U 1,000 U	(2.5 U/㎕)	551-025 551-050 551-100
Clean α-Taq DNA polymerase	250 U 500 U 1,000 U	(2.5 U/㎕)	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.

Ordering Information

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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