Ver 2.2

Ribospin™ vRD

VIRAL RNA/DNA PURIFICATION HANDBOOK

REF 302-150/302-103

HB3200

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CE IVD

GeneAll

Customer & Technical Support

Should you have any further questions, do not hesitate to contact us.

We appreciate your comments and advice.

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Visit GeneAll® Community

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www.geneall.co.kr

Trademarks

This protocol handbook is included in :

GeneAll® Ribospin™ vRD (302-150, 203-103)

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

Used symbols and Markings

REF	Catalog number	IVD	In vitro diagnostic medical device
LOT	Batch number	H B	Handbook code
	Use by	i	Consult instruction for use
	Manufacturer information	Σ <N>	Contains sufficient for <N> tests
	Do not reuse		Temperature limitation
	Production date	EC REP	European Authorized Representative
i	Important note	CONC	Contains the concentrated solution. Additional material must be added before use
? EtOH _____	Write down the current date after adding ethanol to the bottle	EtOH ?	Mark up after adding ethanol

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Kit Contents

Components	Quantity		Storage
Cat. No.	302-150	302-103	
No. of preparation	50	300	Room temperature (15~25°C)
Buffer VL	30 ml	170 ml	
Buffer RB1(concentrate) *	8 ml	48 ml	
Buffer RBW (concentrate) *	13 ml	77 ml	
Buffer RNW (concentrate) * †	6 ml	34 ml	
Nuclease-free water	15 ml	20 ml	
Mini column type V (with collection tube)	50	300	
1.5 ml microcentrifuge tube	50	300	
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** 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

Product Specifications

Ribospin™ vRD	
Type	Using spin column
Maximum volume of starting samples	300 μl/prep
Preparation time	20 min
Maximum loading volume	800 μl
Minimum elution volume	30 μl

Quality Control

All components of GeneAll® Ribospin™ vRD are manufactured in strictly clean conditions, and their its degree of cleanliness is monitored periodically.
To maintain consistency, a quality control process is carried out thoroughly from lot to lot and only the qualified kits are approved for delivery according to ISO 9001:2008 and EN ISO13485:2012.

Storage Conditions

All components of GeneAll® Ribospin™ vRD should be stored at room temperature (15~25°C).
During shipment or storage under cool ambient condition, a precipitate can form in buffer VL. In such a case, heat the bottle to 56°C to dissolve completely. GeneAll® Ribospin™ vRD is guaranteed until the expiration data printed on the product box.

Safety Information

The buffers included in the GeneAll® Ribospin™ vRD 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.

⚠ Buffer VL, RB1, and RBW contain chaotropes, which 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 during RNA purification. Wear disposable gloves always, because skin often contains bacteria and molds 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

GeneAll® Ribospin™ vRD provides a convenient method for isolation of RNA and DNA from cell-free fluid, cell-culture medium, plasma, serum, swab, urine and virus-infected samples.

GeneAll® Ribospin™ vRD utilizes the glass fiber membrane technology for the fastest and the most convenient nucleic acid isolation as a sufficient level for downstream application instead of conventional alcohol precipitation or phenol/chloroform extraction.

The buffer system of Ribospin™ vRD provides the effective binding condition of RNA and DNA to glass fiber membrane and the impurities on the membrane are washed away by two different wash buffers. At least, pure RNA and DNA are eluted in Nuclease-free water. The whole procedure may take only 15 minutes at room temperature and the eluate is suitable for PCR, RT-PCR, or any downstream application without further manipulation. The purified nucleic acid 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 after extraction, it is recommended to store at 4°C for immediate analysis or to freeze at -70°C for long-term storage.

PROTOCOL FOR

Ribospin™ vRD

Equipment and reagents to be supplied by user

* Ethanol (>99%, ACS grade or better)
* 1.5 ml microcentrifuge tubes
* Micropipettes and sterile pipet tips
* Centrifuge capable of attaining 10,000 x g
* Vortex mixer

- Ethanol (>99%, ACS grade or better) must be added before the first use of buffer RB1, RBW and RNW. Please refer to the information on the label of each bottle.
- If a precipitate is formed in buffer VL, heat to 56°C to dissolve completely before use.

1. **Transfer up to 300 μl sample (swab-storage media, cell-free fluid, cell-culture supernatant, plasma, serum, urine) in 1.5 ml microcentrifuge tube.**

2. **Add 500 μl buffer VL to the tube and lyse the sample by pipetting or vortexing.**
   The volume of buffer VL can be adjusted in proportion to the volume of sample.
   For proper lysis, the complete mixing of sample and buffer VL is essential.

3. **Incubate the lysate for 10 min at room temperature.**
   After this step, briefly centrifuge the tube to remove drops from the inside of the lid.

4. **Add 700 μl buffer RB1 to the lysate and mix thoroughly by inverting or vortexing.**
   The volume of buffer RB1 can be adjusted in proportion to the volume of lysate.
   * Do NOT centrifuge at this step.

5. **Transfer up to 750 μl of the mixture to a mini column type V.**

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

7. **Repeat step 5~6 with the remainder of the sample.**
   Discard the pass-through and reinsert the mini column back into the same tube.

8. **Add 500 μl buffer RBW to the mini column.**

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

10. **Add 500 μl buffer RNW to the mini column.**

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

12. **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 carry-over buffer RNW.
    If the carry-over buffer RNW still occurs, centrifuge again for 1min at full speed before transferring the column to the new 1.5 ml microcentrifuge tube.

13. **Add 30~50 μl of Nuclease-free water to the center of the membrane in the mini column.**
    Let it stand for 1 min.

14. **Centrifuge at ≥10,000 x g for 1 min at room temperature.**
    Purified nucleic acid can be stored at 4°C for immediate analysis or at -70°C for long-term storage.

Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low yield	**Poor quality of starting material**	Fresh sample or well-conserved sample should be used for good result. Repeated freezing and thawing the sample should be avoided.
	Low concentration of viral particle in the starting sample	Use more starting sample. If the amount of sample is more than 300 μl, concentrate the volume to 300 μl using a micro-concentrator.
	Inefficient or insufficient lysis	Be sure to incubate for 10 minutes at room temperature after adding buffer VL. For proper lysis, the complete mixing of the sample and buffer VL is essential.
	Improper elution	Add Nuclease-free water to the center of the mini column membrane and perform incubation for 1 minute before centrifugation.
	Precipitate in buffer VL	A precipitate can be formed in buffer VL at cool ambient temperature. It is because the buffer VL is saturated and its solubility would be reduced at low temperature. Before experiment, any precipitate in the buffer VL should be dissolved completely by heating the buffer at 56°C or above until it disappears.
	Degradation of RNA	RNase can be introduced during purification of nucleic acid. Be certain not to introduce any RNases during the procedure of later handling. Keep tubes closed whenever possible during the extraction and use RNase-free products with sterile and disposable plastic ware.
	Buffer RB1, RBW, or RNW was prepared incorrectly	Check that the concentrated buffer RB1, RBW, and RNW were diluted with the correct volume of absolute ethanol.

Troubleshooting Guide

Facts	Possible Causes	Suggestions
Purified nucleic acid does not perform well in down-stream application	**Residual ethanol from buffer RNW remains in eluate**	Care must be taken for eliminating the carry-over buffer RNW before elution step. The membrane of mini column should be kept completely dry via additional centrifugation (Step 12, page 9) or air-drying.
	Incorrect order of buffer RBW and RNW	Ensure that buffer RBW and RNW are used in the correct order during extraction. If used in the wrong order, perform the last washing step with buffer RNW.

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*

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
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/㎕)
		500 U	501-050	
		1,000 U	501-100	
α-Taq DNA polymerase		250 U	502-025	(2.5 U/㎕)
		500 U	502-050	
		1,000 U	502-100	
α-Pfu DNA polymerase		250 U	504-025	(2.5 U/㎕)
		500 U	504-050	
		1,000 U	504-100	
Fast-Pfu DNA polymerase		250 U	505-025	(2.5 U/㎕)
		500 U	505-050	
		1,000 U	505-100	
Hotstart Taq DNA polymerase		250 U	531-025	(2.5 U/㎕)
		500 U	531-050	
		1,000 U	531-100	
Taq Premix	96 tubes	20 ㎕	521-200	lyophilized
		50 ㎕	521-500	
		20 ㎕	526-200	solution
		50 ㎕	526-500	
α-Taq Premix	96 tubes	20 ㎕	522-200	lyophilized
		50 ㎕	522-500	
		20 ㎕	527-200	solution
		50 ㎕	527-500	
HS-Taq Premix	96 tubes	20 ㎕	525-200	solution
		50 ㎕	525-500	
		20 ㎕	520-200	lyophilized
α-Pfu Premix	96 tubes	50 ㎕	523-500	solution
Taq Premix (w/o dye)	96 tubes	20 ㎕	524-200	lyophilized
dNTPs mix		500 ㎕	509-020	2.5 mM each
dNTPs set (set of dATP, dCTP, dGTP and dTTP)		1 ml x 4 tubes	509-040	100 mM

Products	Scale	Size	Cat. No.	Type

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
α-Taq Master mix	0.5 ml x 2 tubes	542-010	solution
	0.5 ml x 10 tubes	542-050	solution
HS-Taq Master mix	0.5 ml x 2 tubes	545-010	solution
	0.5 ml x 10 tubes	545-050	solution
α-Pfu Master mix	0.5 ml x 2 tubes	543-010	solution
	0.5 ml x 10 tubes	543-050	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
RT Master mix with oligo $(dT)_{20}$	0.5 ml × 2 tubes	601-730	solution
RT Master mix with random hexamer	0.5 ml × 2 tubes	601-740	solution
RT Premix	96 tubes, 20 μℓ	601-602	solution
RT Premix with oligo $(dT)_{20}$	96 tubes, 20 μℓ	601-632	solution
RT Premix with random hexamer	96 tubes, 20 μℓ	601-642	solution
One-step RT-PCR Master mix	0.5 ml × 2 tubes	602-110	solution
One-step RT-PCR Premix	96 tubes, 20 μℓ	602-102	solution
First strand Synthesis Kit	50 reaction	605-005	solution
ZymAll™ RNase Inhibitor	10,000 U	605-010	solution
ZymAll™ RNase Inhibitor	4,000 U	605-004	solution

GeneAll® **RealAmp™** *for qPCR amplification*

SYBR qPCR Master mix (2X, Low ROX)	200 rxn 20 μℓ	801-020	solution
	500 rxn 20 μℓ	801-050	
SYBR qPCR Master mix (2X, High ROX)	200 rxn 20 μℓ	801-021	solution
	500 rxn 20 μℓ	801-051	

Products	Size	Cat. No.

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*

STEADi™	12 Instrument		GST012
STEADi™	24 Instrument		GST024
STEADi™	Genomic DNA Cell / Tissue	96	401-104
STEADi™	Genomic DNA Blood	96	402-105
STEADi™	Bacteria DNA	96	403-106
STEADi™	Total RNA	96	404-304
STEADi™	Viral DNA / RNA	96	405-322
STEADi™	CFC Seed DNA / RNA	96	406-C02

NOTE

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