HB3220

Ribospin™ vRD II

VIRAL RNA PURIFICATION HANDBOOK

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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This protocol handbook is included in :

GeneAll® Ribospin™ vRD II (322-150, 322-103)

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

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Kit Contents

Cat. No.	322-150	322-103	Storage
Components	Quantity		
Buffer NVL	16 ml	100 ml	Room temperature (15~25°C)
Buffer RB1 (concentrate) *	5 ml	25 ml	
Buffer RBW (concentrate) *	13 ml	77 ml	
Buffer RNW (concentrate) * †	6 ml	34 ml	
Nuclease-free water	15 ml	20 ml	
Carrier RNA **	370 μg	2.3 mg	
Micro column type S (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

*** Refer to page 7 for instruction of Carrier RNA*

Product Specifications

Ribospin™ vRD II	
Type	Spin
Maximum amount of starting samples	100 μl/prep
Preparation time	~15 min
Maximum loading volume	750 μl
Minimum elution volume	20 μl

Quality Control

All components in GeneAll® Ribospin™ vRD II are manufactured in strictly clean conditions, and its degree of cleanness is monitored periodically. Quality control is carried out thoroughly from lot to lot, and only the qualified kits are approved to be delivered.

Storage Conditions

All components of GeneAll® Ribospin™ vRD II should be stored at room temperature (15~25°C). After reconstitution of Carrier RNA with Nuclease-free water, the Carrier RNA solution should be stored at -20°C in aliquots for conservation of activity or used immediately for experiments.

During shipment or storage under cool ambient condition, a precipitate can form in buffer NVL. In such a case, heat the bottle to 56°C to dissolve completely. GeneAll® Ribospin™ vRD II is guaranteed until the expiration data printed on the product box.

Safety Information

Buffer NVL, RB1, and RBW contain an irritant which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken when handling such material. Always wear gloves and eye protection, and follow standard safety precautions.

Buffer NVL contains 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 of 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

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

The GeneAll® Ribospin™ vRD II utilizes the glass fiber membrane technology to purify nucleic acid as a sufficient level for downstream analysis instead of conventional alcohol precipitation or phenol/chloroform extraction.

The buffer system of Ribospin™ vRD II 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 last, pure RNA and DNA are eluted in Nuclease-free water. Whole procedure takes only 15 minutes at room temperature and the purified nucleic acid 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 labware 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.

Before Experiment

Freshly harvested samples should be used or stored immediately for the best result. Starting material, such as plasma or serum, should be stored at -70°C in aliquots for long-term storage.
Repeated freezing and thawing the samples leads to protein precipitation that may cause decreased yield of the extracted viral nucleic acid due to reduction of viral titers in the sample. Besides, the protein precipitant will cause clogging of spin column.
The GeneAll® Ribospin™ vRD II is designed for extraction of total nucleic acids from samples including virus and host cell. The use of cell-free body fluids is recommended for isolation of viral nucleic acid, and the extraction efficiency can vary depending on the type of virus and sample media.

Carrier RNA

This kit provides Carrier RNA, which can add at lysis step if required. Provided Carrier RNA can help to improve the binding capacity of mini spin column when viral nucleic acids included in sample are low-copy and protect target nucleic acids from the chance of degradation due to residual RNase activity.
For purification of nucleic acid from very few target molecules in sample, we recommend adding Carrier RNA at lysis step. To obtain a solution of 1 μg/μl, add 370 μl (Cat. No. 322-150) or 2.3 ml (Cat. No. 322-103) of Nuclease-free water to the tube containing lyophilized Carrier RNA. Dissolve the Carrier RNA thoroughly, divide it into conveniently sized aliquots, and store at -20°C. Do not freeze-thaw the aliquots of Carrier RNA more than 3 times. For one preparation, 7 μl of dissolved Carrier RNA is required.

PROTOCOL FOR

Ribospin™ vRD II

Before experiment

- *Before first use, add absolute ethanol (ACS grade or better) into buffer RB1, RBW and RNW as indicated on the bottle.*
- *If a precipitate is formed in buffer NVL, heat to 56°C to dissolve before use.*
- *Prepare an aliquot of Carrier RNA for use on ice (Refer to page 7 for instruction of Carrier RNA).*

1. **Add 300 µl of buffer NVL and 7 µl of Carrier RNA solution into a 1.5 ml microcentrifuge tube.**

2. **Transfer up to 100 µl of sample into the 1.5 ml microcentrifuge tube.**
   If the sample volume is less than 100 µl, adjust the volume to 100 µl with 1X PBS.
   In case of large sample volume, increase the amount of buffer NVL and Carrier RNA solution proportionally.

3. **Mix thoroughly by vortexing for 10 sec.**
   For proper lysis, the complete mix of sample and buffer NVL is essential.

4. **Incubate the mixture for 10 min at room temperature.**

5. **Add 350 µl of buffer RB1 to the mixture and mix thoroughly by vortexing for 10 sec.**
   The volume of buffer RB1 can be adjusted in proportion to the volume of lysate.
   Normally, the ratio of buffer RB1 to the mixture is 1:1.
   Do not centrifuge at this step because nucleic acids can be precipitated through centrifugation.

6. **Transfer up to 750 µl of the mixture to a spin column (Micro column type S, white).**

7. **Centrifuge at ≥10,000 x g for 30 sec at room temperature.**
   Discard the pass-through and reinsert the spin column back into the same tube.
   If the mixture volume exceeds 750 μl, repeat step 6~7 with the remainder.

8. **Add 500 μl of buffer RBW to the spin column.**

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

10. **Add 500 μl of buffer RNW to the spin column.**

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

12. **Centrifuge at full speed for an additional 1 min at room temperature to remove residual wash buffer.**
    Transfer the spin column to a new 1.5 ml microcentrifuge tube (provided).
    Care must be taken at this step for eliminating the carry-over of buffer RNW that can interfere with downstream reactions.
    If a carry-over of buffer RNW still occurs, centrifuge again for 1 min at full speed with the collection tube before transferring to the new 1.5 ml microcentrifuge tube.

13. **Add 20~50 μl of Nuclease-free water to the center of the membrane in the spin column.**
    Let it stand for 1 min.

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

Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low yield	**Poor quality of starting material**	Too old or improperly stored sample often yield degraded DNA. Use fresh sample, if possible. Repeated freezing and thawing the sample should be avoided.
	Low concentration of viral particle in the starting sample	Use more the starting sample. If the amount of sample is more than 300 μl, concentrate the volume to 300 μl using a microconcentrator.
	Inefficient or insufficient lysis	Be sure to incubate for 10 min at room temperature after adding buffer NVL. For proper lysis, the complete mix of sample and buffer NVL is essential.
	Improper elution	Add Nuclease-free water to the center of the spin column membrane and perform incubation for 1 min before centrifugation.
	Precipitation of buffer NVL	Storage at cool ambient temperature may cause precipitation in buffer NVL. For a good result, any precipitate in the buffer should be dissolved 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 or later handling. Keep tubes closed whenever possible during the extraction and use RNase-free products with sterile and disposable plastic ware.

Troubleshooting Guide

Facts	Possible Causes	Suggestions
	Incorrect use of Carrier RNA solution	Add Carrier RNA solution at lysis step. Omission of Carrier RNA may lead to low purification efficiency.
	Degradation of Carrier RNA	Carrier RNA should be stored at -20°C in aliquots after reconstitution. Do not freeze-thaw the aliquots of Carrier RNA more than 3 times.
Purified nucleic acid does not perform well in downstream application	**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.
	Residual ethanol from buffer RNW remains in eluate	Care must be taken for eliminating the carry-over of buffer RNW before elution step. The membrane of mini spin column should be kept completely dry via additional centrifugation or air-drying.

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	
		1,000	101-111	
	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/μℓ)
		500 U	501-050	
		1,000 U	501-100	
α-Taq DNA polymerase		250 U	502-025	(2.5 U/μℓ)
		500 U	502-050	
		1,000 U	502-100	
α-Pfu DNA polymerase		250 U	504-025	(2.5 U/μℓ)
		500 U	504-050	
		1,000 U	504-100	
Fast-Pfu DNA polymerase		250 U	505-025	(2.5 U/μℓ)
		500 U	505-050	
		1,000 U	505-100	
Hotstart Taq DNA polymerase		250 U	531-025	(2.5 U/μℓ)
		500 U	531-050	
		1,000 U	531-100	
Taq Premix	96 tubes	20 μℓ	521-200	lyophilized
		50 μℓ	521-500	
		20 μℓ	526-200	solution
		50 μℓ	526-500	
α-Taq Premix	96 tubes	20 μℓ	522-200	lyophilized
		50 μℓ	522-500	
		20 μℓ	527-200	solution
		50 μℓ	527-500	
HS-Taq Premix	96 tubes	20 μℓ	525-200	solution
		50 μℓ	525-500	
		20 μℓ	520-200	lyophilized
α-Pfu Premix	96 tubes	50 μℓ	523-500	solution
Taq Premix (w/o dye)	96 tubes	20 μℓ	524-200	lyophilized
dNTPs mix		500 μℓ	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*			
12 Instrument		GST012	system
24 Instrument		GST024	system
Genomic DNA Cell / Tissue	96	401-104	kit
Genomic DNA Blood	96	402-105	kit
Bacteria DNA	96	403-106	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	407-107	kit
Soil DNA	96	407-108	kit

NOTE

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