

Cat.No. 322-150

RibospinTM vRD II

VIRAL 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® Ribospin™ vRD II (322-150)

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

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KIT CONTENTS

Components	Quantity	Storage
Buffer NVL	16 ml	Room temperature (15~25°C)
Buffer RB1	22 ml	
Buffer RBW	30 ml	
Buffer RNW	30 ml	
Nuclease-free water	15 ml	
Carrier RNA*	370 µg	
Column micro S with collection tube	50 ea	
1.5 ml microcentrifuge tube	50 ea	

* Refer to page 7 for carrier RNA

Product Specifications

Ribospin™ vRD II

Type	Spin
Maximum volume of starting samples	100 µl / prep
Preparation time	~ 15 minutes
Maximum loading volume	750 µl
Minimum elution volume	20 µl

Quality control

All components in Ribospin™ vRD II are 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

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, it should be stored in aliquots at -20°C for conservation of activity or immediately used for experiments.

Under cool ambient condition, a precipitate can be formed in buffer NVL. In such a case, heat the bottle above 37°C to dissolve completely. GeneAll Ribospin™ vRD II is guaranteed until the expiration date printed on the product label.

Precautions

Buffer NVL, RB1, and RBW 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

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

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

Ribospin™ vRD II procedures employed the glassfiber membrane technology for the fastest and the most convenient of high purity RNA and DNA isolation, instead of conventional alcohol precipitation or phenol/chloroform extraction.

Ribospin™ vRD II buffer system provides the effective binding condition of RNA and DNA to glassfiber membrane and the impurities on the membrane are washed away by two different wash buffers. At last, pure RNA and DNA are eluted by nuclease-free water. Whole procedure takes only 15 minutes and the purified nucleic acid is suitable for PCR, RT-PCR, or any downstream application without further manipulation.

Ribospin™ vRD II procedure should be performed at room temperature. 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, it is recommended to store at 4°C for immediate analysis or to freeze at -70°C for long-term storage.

Before Experiment

Starting material, such as plasma or serum, should be stored at -70°C in aliquots for long term storage. Repeated freezing and thawing of frozen plasma or serum leads to protein precipitation, causing reduced viral titers and subsequently decreased yields of the isolated viral nucleic acid. Besides, protein precipitant will cause clogging of spin column.

Ribospin™ vRD II is designed to extract 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.

Provided carrier RNA can help to improve the binding of viral nucleic acids to the spin column especially in the case of very few target nucleic acids in the samples, and protect target nucleic acids from the chance of degradation due to residual RNase activity.

Carrier RNA

This kit is provided with carrier RNA, which can be added at lysis step if required. Carrier RNA enhances binding of nucleic acid to the spin column membrane, especially if there are very few target molecules in the sample.

For purification of nucleic acid from very small amounts of sample, we recommend adding carrier RNA at lysis step. To obtain a solution of 1 ug/ul, add 370 ul of nuclease-free water to the tube containing 370 ug 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, 7ul of dissolved carrier RNA is required.

PROTOCOL FOR

Ribospin™ vRD II

1. Add 300 ul of buffer NVL and 7 ul of carrier RNA into a tube.

2. Transfer upto 100 ul of serum sample into the tube.

If the sample volume is less than 100 ul, adjust the volume to 100 ul with PBS.

In case of large sample volume, increase the amount of buffer NVL and carrier RNA proportionally.

3. Mix thoroughly by vortexing for 10 seconds.

For proper lysis, the complete mix of sample and buffer NVL is essential.

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

5. Add 350 ul of buffer RB1 to the mixture and mix thoroughly by vortexing for 10 seconds.

The volume of buffer RB1 can be adjusted in proportion to the volume of lysate.

Do not centrifuge at this step. Nucleic acids can be precipitated through centrifugation.

6. Transfer upto 750 ul of the mixture to a spin column (Column type micro S, white).

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

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

If the sample volume exceeds 750 ul, repeat step 6 ~ 7 with the remainder of the sample.

- 8. Add 500 ul of buffer RBW to the spin column.**
- 9. Centrifuge at $\geq 10,000 \times g$ for 30 seconds at room temperature**
Discard the pass-through and reinsert the spin column back into the same tube.
- 10. Add 500 ul of buffer RNW to the spin column.**
- 11. Centrifuge at $\geq 10,000 \times g$ for 30 seconds 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 minute at room temperature to remove residual wash buffer.**
Transfer the 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.
- 13. Add 25 ~ 50 ul of nuclease-free water to the center of the membrane in the spin column.**
Let it stand for 1 minute.
- 14. Centrifuge at $\geq 10,000 \times g$ for 1 minute at room temperature.**
Purified nucleic acids can be stored at 4°C for immediate analysis and can be stored at -70°C for long term storage.

■ Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low yield	Poor quality of starting material	Repeated freezing and thawing should be avoided.
	Low concentration of virus in the sample	Use more sample. Concentrate the sample volume to 300 ul using a microconcentrator.
	Sample not homogenized completely	Be sure to incubate for 10 minutes at room temperature after lysis. For proper lysis, the complete mix of sample and buffer NVL is essential.
	Incorrect elution conditions	Add nuclease-free water to the center of the spin column membrane and perform incubation for 1 minute before centrifugation.
	Precipitation of buffer NVL	Storage at low temperature may cause precipitation in buffer NVL. For good result, any precipitate in the buffer should be dissolved completely by incubating the buffer at 37°C(or above) until it disappears.
	Degradation of RNA	RNase can be introduced during use. Be certain not to introduce any RNases during the procedure or later handling. Keep tubes closed whenever possible during the preparation.

■ Troubleshooting Guide

Facts	Possible Causes	Suggestions
	Carrier RNA not added	Add carrier RNA at lysis step. Omission of carrier RNA leads to low purification efficiency.
	Degradation of carrier RNA	Carrier RNA was not stored at -20°C or afflicted with multifold freeze-thaw cycles. After reconstitution, carrier RNA should be stored in aliquots at -20°C.
	Buffer RBW and RNW used in the wrong order	Ensure that buffer RBW and RNW are used in the correct order in the protocol. If used in the wrong order, perform the last washing step with RNW.
Eluate does not perform well in downstream application	Residual ethanol remains in eluate	To remove any residual ethanol included in buffer RNW from spin column membrane, centrifuge again for complete removal of ethanol (step 12).
	Buffer RBW and RNW used in the wrong order	Ensure that buffer RBW and RNW are used in the correct order in the protocol. If used in the wrong order, perform the last washing step with RNW.

Ordering Information

Products	Scale	Size	Cat. No.	Type
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GeneAll® Hybrid-Q™ for rapid preparation of plasmid DNA

Plasmid Rapidprep	50	100-150	mini / spin
	200	100-102	

GeneAll® Expres™ for preparation of plasmid DNA

Plasmid SV	mini	50	101-150	spin / vacuum
		200	101-102	
		1,000	101-111	
Midi	Midi	26	101-226	spin / vacuum
		50	101-250	
		100	101-201	

GeneAll® Exfection™ for preparation of highly pure plasmid DNA

Plasmid LE (Low Endotoxin)	mini	50	111-150	spin / vacuum
		200	111-102	
		26	111-226	
Midi	Midi	100	111-201	vacuum
		20	121-220	
Plasmid EF (Endotoxin Free)	Midi	100	121-201	spin

GeneAll® Expin™ for purification of fragment DNA

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

Tissue SV	mini	100	104-101	spin / vacuum	
		250	104-152		
	Midi	Midi	26	104-226	spin / vacuum
			100	104-201	
Tissue plus! SV	MAXI	10	104-310	spin / vacuum	
		26	104-326		
	mini	mini	100	109-101	spin / vacuum
			250	109-152	
Midi	Midi	26	109-226	spin / vacuum	
		100	109-201		
	MAXI	MAXI	10	109-310	spin / vacuum
			26	109-326	

Products	Scale	Size	Cat. No.	Type
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GeneAll® Exgene™ for isolation of total DNA

Blood SV	mini	100	105-101	spin / vacuum	
		250	105-152		
	Midi	Midi	26	105-226	spin / vacuum
			100	105-201	
Cell SV	MAXI	10	105-310	spin / vacuum	
		26	105-326		
	mini	mini	100	106-101	spin / vacuum
			250	106-152	
Clinic SV	MAXI	10	106-310	spin / vacuum	
		26	106-326		
	mini	mini	100	108-101	spin / vacuum
			250	108-152	
Genomic DNA micro	Midi	26	108-226	spin / vacuum	
		100	108-201		
	MAXI	MAXI	10	108-310	spin / vacuum
			26	108-326	
Plant SV	mini	50	118-050	spin	
		100	117-101		
	Midi	Midi	250	117-152	spin / vacuum
			26	117-226	
Soil DNA mini	MAXI	10	117-310	spin / vacuum	
		26	117-326		
	mini	mini	50	114-150	spin
			200	107-150	
GMO SV	mini	50	107-150	spin / vacuum	
		200	107-102		

GeneAll® GenEx™ for isolation of total DNA

GenEx™ Blood	Sx	100	220-101	solution	
		500	220-105		
GenEx™ Cell	Lx	100	220-301	solution	
		500	221-105		
GenEx™ Tissue	Sx	100	221-101	solution	
		500	221-105		
	Lx	Lx	100	221-301	solution
			500	222-101	
Lx	Lx	100	222-105	solution	
		500	222-301		

Products	Scale	Size	Cat. No.	Type
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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 preparation 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 preparation 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™ vRD	mini	50	302-150	spin
Ribospin™ vRD plus!	mini	50	312-150	spin
Ribospin™ Plant	mini	50	307-150	spin
Allspin™	mini	50	306-150	spin

Products	Scale	Size	Cat. No.	Type
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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	
Pfu DNA polymerase		250 U	503-025	(2.5 U/μℓ)
		500 U	503-050	
		1,000 U	503-100	
α-Pfu DNA polymerase		250 U	504-025	(2.5 U/μℓ)
		500 U	504-050	
		1,000 U	504-100	
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	
		50 μℓ	526-500	
α-Taq Premix	96 tubes	20 μℓ	522-200	lyophilized
		50 μℓ	522-500	
		20 μℓ	527-200	
		50 μℓ	527-500	
HS-Taq Premix	96 tubes	20 μℓ	525-200	solution
		50 μℓ	525-500	
Taq Premix (w/o dye)	96 tubes	20 μℓ	524-200	lyophilized
		50 μℓ	524-500	
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

* Each dNTPs is available

Products	Scale	Size	Cat. No.	Type
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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

Products	Scale	Size	Cat. No.	Type
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GeneAll® HyperScript™ for Reverse Transcription

Reverse Transcriptase	10,000 U	601-100	(200 U/ μ l)
RT Master mix	0.5 ml x 2 tubes	601-710	solution
RT Premix	96 tubes, 20 μ l	601-602	solution
Onestep RT-PCR Master mix	0.5 ml x 2 tubes	602-110	solution
Onestep RT-PCR Premix	96 tubes, 20 μ l	602-102	solution

NOTE



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