Ver 1.0

Handbook for

Viral DNA/RNA

exgene™

DNA 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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Trademarks

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This protocol handbook is included in :

GeneAll® Exgene™ Viral DNA/RNA kit (128-150)

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

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KIT CONTENTS

GeneAll® Exgene™ Viral DNA/RNA kit

Cat. No. 128-150

Components	Quantity	Storage
Buffer BL	15 ml	Room temperature (15~25°C)
Buffer RB1	22 ml	
Buffer BW	30 ml	
Buffer TW	50 ml	
Nuclease-free water	15 ml	
Proteinase K *	13 mg	
PK Storage bfr.*	1 ml	
Carrier RNA **	370 ug	
Column micro S with collection tube	50 ea	
1.5 ml microcentrifuge tube	50 ea	

* Refer to page 9 for Proteinase K

** Refer to page 8 for carrier RNA

Product Specifications

Exgene™ Viral DNA/RNA kit	
Type	Spin
Maximum volume of starting samples	200 ul / prep
Preparation time	~ 20 minutes
Maximum loading volume	750 ul
Minimum elution volume	20 ul

Quality Control

All components in GeneAll® Exgene™ Viral DNA/RNA kit 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® Exgene™ Viral DNA/RNA kit should be stored at room temperature (15~25°C). After reconstitution of proteinase K with storage buffer, it should be stored under 4°C for conservation of activity. It can be stored at 4°C for 1 year without significant decrease in activity. But for prolonged preservation of activity, storing under -20°C is recommended. Also, dissolved carrier RNA should be immediately used for experiments or frozen in aliquots at -20°C.
Under cool ambient condition, a precipitate can be formed in buffer BL. In such a case, heat the bottle above 37°C to dissolve completely. GeneAll® Exgene™ Viral DNA/RNA kit is guaranteed until the expiration date printed on the product label.

Precautions

Buffer BL, RB1, and BW 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

The Exgene™ Viral DNA/RNA kit provides fast and easy methods for the purification of total nucleic acids from viral samples such as cell-free fluid, cell-cultrue supernatant, plasma, serum, swab, urine, and virus-infected samples. Purified nucleic acids can be used directly for PCR, qPCR, RT-PCR, or any downstream application without further manipulation.

Exgene™ Viral DNA/RNA kit utilizes the advanced silica-binding technology to purify total nucleic acids sufficiently pure for many applications. Viral samples are lysed in optimized buffer containing detergent and lytic enzyme. Under optimized binding condition, nucleic acids in the lysate bind to silica membrane and impurities pass through membrane into a collection tube. The membranes are washed with a series of alcohol-containing buffer to remove any traces of proteins, cellular debris and salts. Finally pure nucleic acids are released into a clean collection tube with deionized water or low ionic strength buffer. The eluate should be treated with care because nucleic acids are very sensitive to contaminants, such as nucleases, often found on general labware and dust. To ensure nucleic acids 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.

Exgene™ Viral DNA/RNA kit 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 it can also 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 to the 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.

Proteinase K

This kit provides Proteinase K and PK storage buffer for dissolving proteinase K. Reconstituted proteinase K serves efficient viral lysis for most sample types.

To obtain a solution of 20 mg/ml, add 650 ul of PK storage buffer to the tube of lyophilized proteinase K, and mix carefully to avoid foaming.

After reconstitution of proteinase K with PK storage buffer, it should be stored under 4°C for conservation of activity. It can be stored at 4°C for 1 year without significant decrease in activity. But for prolonged preservation of activity, storing under -20°C is recommended.

Exgene™ Viral DNA/RNA kit Protocol

1. **Pipet 10 ul of proteinase K solution into the bottom of a 1.5 ml microcentrifuge tube.**

2. **Transfer upto 200 ul of sample to the tube.**
   If the sample volume is less than 200 ul, adjust the volume to 200 ul with PBS.

3. **Add 200 ul of buffer BL to the tube.**
   In case of large sample volume, increase the amount of buffer BL and carrier RNA proportionally.

4. **Add 7 ul of carrier RNA to the tube and mix thoroughly by vortexing for 10 seconds**
   It is essential to mix the sample and buffer BL thoroughly for good result.

5. **Incubate the tube at 56°C for 10 minutes.**
   Spin down briefly to remove any drops from inside of the lid.

6. **Add 400 ul of buffer RB1 to the sample 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.

7. **Transfer the mixture to the spin column carefully (Column type micro S, white).**

8. Centrifuge at ≥ 10,000 x g for 1 minute 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 7 ~ 8 with the remainder of the sample.

9. Add 500 ul of buffer BW to the spin column.

10. Centrifuge at ≥ 10,000 x g for 1 minute at room temperature.
Discard the pass-through and reinsert the spin column back into the same tube.

11. Add 700 ul of buffer TW to the spin column.

12. Centrifuge at ≥ 10,000 x g for 1 minute at room temperature.
Discard the pass-through and reinsert the spin column back into the same tube.

13. Centrifuge at full speed for 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 TW.

14. **Add 20 ~ 50 ul of nuclease-free water to the center of the membrane in the spin column.**
Let it stand for 1 minute.

15. **Centrifuge at ≥ 10,000 x 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.

Trouble Shooting

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	For proper lysis, the complete mix of sample and buffer BL 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 BL	Storage at low temperature may cause precipitation in buffer BL. 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.
	Carrier RNA not added	Add carrier RNA at lysis step. Omission of carrier RNA leads to low purification efficiency.

Facts	Possible Causes	Suggestions
	Degradation of carrier RNA	Carrier RNA was not stored at -20°C or afflicted with multiful freeze-thaw cycles. After reconstitution, carrier RNA should be stored in aliquots at -20°C.
	Buffer BW and TW used in the wrong order	Ensure that buffer BW and TW are used in the correct order in the protocol. If used in the wrong order, perform the last washing step with TW.
Eluate does not perform well in downstream application	Residual ethanol remains in eluate	To remove any residual ethanol included in buffer TW from spin column membrane, centrifuge again for complete removal of ethanol (step 13).
	Buffer BW and TW used in the wrong order	Ensure that buffer BW and TW are used in the correct order in the protocol. If used in the wrong order, perform the last washing step with TW.

Ordering Information

GeneAll® *Hybrid-Q™* *for rapid preparation of plasmid DNA*

Products	Scale	Size	Cat. No.	Type
Plasmid Rapidprep		50	100-150	mini / 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 highly pure 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
GMO SV	mini	50	107-150	spin / vacuum
		200	107-102	

GeneAll® *GenEx™* *for isolation of total DNA*

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 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
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	
Pfu DNA polymerase		250 U	503-025	(2.5 U/㎕)
		500 U	503-050	
		1,000 U	503-100	
α-Pfu DNA polymerase		250 U	504-025	(2.5 U/㎕)
		500 U	504-050	
		1,000 U	504-100	
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
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

* Each dNTPs is available

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

Products	Scale	Size	Cat. No.	Type
GeneAll® *HyperScript™* *for Reverse Transcription*				
Reverse Transcriptase		10,000 U	601-100	(200 U/μℓ)
RT Master mix	0.5 ml × 2 tubes		601-710	solution
RT Premix	96 tubes,	20 μℓ	601-602	solution
Onestep RT-PCR Master mix	0.5 ml × 2 tubes		602-110	solution
Onestep RT-PCR Premix	96 tubes,	20 μℓ	602-102	solution

NOTE

GeneAll

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