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Handbook for Viral DNA/RNA



DNA 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[®] 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

Cat. No. 128-150		
Components	Quantity	Storage
Buffer BL	l 5 ml	
Buffer RB1	22 ml	
Buffer BW	30 ml	
Buffer TW	50 ml	5
Nuclease-free water	l 5 ml	Koom
Proteinase K *	13 mg	temperature
PK Storage bfr.*	l ml	(15~25°C)
Carrier RNA **	370 ug	
Column micro S with collection tube	50 ea	
1.5 ml microcentrifuge tube	50 ea	

$\mathbf{GeneAll}^{\mathbb{R}} \mathbf{Exgene}^{{}^{\mathrm{TM}}} \mathbf{Viral} \ \mathbf{DNA} / \mathbf{RNA} \ \mathbf{kit}$

* Refer to page 9 for Proteinase K

** Refer to page 8 for carrier RNA

Product Specifications

Exgene™ Viral DNA/RNA kit				
Туре	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 I 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[®] ExgeneTM 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

I. 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.
- I 0. 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.
- []. Add 700 ul of buffer TW to the spin column.
- **12.** Centrifuge at \geq 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.

Exgene[™] Viral DNA/RNA kit Protocol

- I4. Add 20 ~ 50 ul of nuclease-free water to the center of the membrane in the spin column.
 Let it stand for 1 minute.
- I 5. 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

Products	Scale	Size	Cat.	No.	Туре	Products	Scale	Size	Cat. No.
GeneAll® Hybrid	I-Q™ for	rapid pr	eparatio	on of p	olasmid DNA	GeneAll® Exgene	г м for iso	olation of	total DNA
Plasmid Rapidprep		50	100-	150				100	105-101
		200	100-	102	mini / spin		mini	250	105-152
						-		26	105-226
GeneAll [®] Expret	5TM for pr	eparatior	n of plas	mid E	DNA	Blood SV	Midi	100	105-201
· ·		50	101-	150		-		10	105-310
	mini	200	101-	102	spin /		MAXI	26	105-326
		1,000	101-		vacuum	· · · · · · · · · · · · · · · · · · ·		100	106-101
Plasmid SV		26	101-2	226		C 11 C) /	mini	250	106-152
	Midi	50	101-2	250	spin /	Cell SV -		10	106-310
		100	101-2	201	vacuum		MAXI	26	106-326
								100	108-101
GeneAll [®] Exfect	ion™						mini	250	108-152
for prepa	ration of	highly pu	re plasn	nid Dl	NA	-	N.C. P.	26	108-226
	mini	50	-	150	spin /	Clinic SV	Midi	100	108-201
Plasmid LE		200	-	102	vacuum	-	MANZI	10	108-310
(Low Endotoxin)	Midi	26		226	spin /		MAXI	26	108-326
		100		201	vacuum	Genomic DNA micro		50	118-050
Plasmid EF	Midi	20	121-2	220	spin			100	7- 0
(Endotoxin Free)	. nai	100	121-2	201	opin		mini	250	7- 52
						-	Midi	26	117-226
GeneAll [®] Expin ^{TI}	Μ for puri	fication o	f fragme	ent DI	VA	Plant SV		100	7-20
Calsy		50	102-	150	spin /		MAXI	10	7-3 0
		200	102-	102	vacuum			26	117-326
		50	103-	150	spin /	Soil DNA mini	mini	50	4- 50
1 CK 3V	TTHEN	200	103-	102	vacuum			50	107-150
Clearly In SV		50	3-	150	spin /	GMO SV	mini	200	107-102
CleanOp SV	TTHEN	200	3-	102	vacuum				
Camba CD	mini	50	2-	150	spin /	GeneAll [®] GenEx [™]	n for isolo	ation of t	otal DNA
COMDO GF	111111	200	2-	102	vacuum			100	220-101
						GenEx TM Blood	Sx	500	220-101
GeneAll® Exgene	e TM for is	olation of	total D	NA			l x	100	220-301
		100	104-	101	spin /		EX	100	221-101
	mini	250	104-	152	vacuum	GenFx [™] Cell	Sx	500	221-105
Time O/	NA: JI	26	104-2	226	spin /		l x	100	221-301
TISSUE 3V	PHO	100	104-2	201	vacuum			100	222-101
		10	104-3	310	spin /	GenFx [™] Tissue	Sx	500	222-105
	MAXI	26	104-3	326	vacuum		l x	100	222-301
		100	109-	101	spin /				222 501
	mini	250	109-	152	vacuum				
Times bl. 101	M	26	109-2	226	spin /				
Tissue plus! SV	I™IIdi	100	109-2	201	vacuum				
	MANZ	10	109-3	310	spin /				
	MAXI	26	109-3	326	vacuum				

spin / vacuum spin spin / vacuum spin / vacuum spin / vacuum spin spin / vacuum

solution solution solution solution solution

Products	Scale	Size	Cat. No.	Туре
			the second s	

GeneAll® GenEx	τ Μ for is	olation of	total DNA	
	Sx	100	227-101	
GenEx [™] Plant	Mx	100	227-201	solution
	Lx	100	227-301	
	Sx	100	228-101	
GenEx™ Plant plus!	Mx	50	228-250	solution
	Lx	20	228-320	

GeneAll[®] DirEx[™] series

for preperation of	for preperation of PCR-template without extraction							
DirEx™	100	250-101	solution					
DirEx [™] <i>Fast-</i> Tissue	96 T	260-011	solution					
DirEx [™] <i>Fast</i> -Cultured cell	96 T	260-021	solution					
DirEx [™] <i>Fast-</i> Whole blood	96 T	260-03 I	solution					
DirEx [™] <i>Fast</i> -Blood stain	96 T	260-041	solution					
DirEx [™] <i>Fast</i> -Hair	96 T	260-051	solution					
DirEx [™] <i>Fast</i> -Buccal swab	96 T	260-061	solution					
DirEx [™] <i>Fast</i> -Cigarette	96 T	260-071	solution					

GeneAll[®] RNA series for preparation of total RNA

RiboEv TM	mini	100	301-001	colution	
NDOLX		200	301-002	SOlUtion	
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		100	302-001	colution	
	TT1IF1I	200	302-002	SOIULION	
Riboclear™	mini	50	303-150	spin	
Riboclear [™] plus!	mini	50	3 3- 50	spin	
Ribospin™	mini	50	304-150	spin	
Ribospin [™] vRD	mini	50	302-150	spin	
Ribospin [™] vRD <i>plus!</i>	mini	50	312-150	spin	
Ribospin [™] Plant	mini	50	307-150	spin	
Allspin [™]	mini	50	306-150	spin	

Products	Scale	Size	Cat. No.	Туре	
GeneAll® AmpO	NE[™] for	PCR arr	plification		
		250 U	501-025		
Taq DNA polymeras	e	500 U	501-050	(2.5 U/ µℓ)	
		U 000, I	501-100		
		250 U	502-025		
lpha-Taq DNA polyme	erase	500 U	502-050	(2.5 ∪/µℓ)	
		U 000, I	502-100		
		250 U	503-025		
Pfu DNA polymerase	9	500 U	503-050	(2.5 ∪/µℓ)	
		I,000 U	503-100		
		250 U	504-025		
lpha-Pfu DNA polyme	rase	500 U	504-050	(2.5 U/µℓ)	
		1,000 U	504-100		
		250 U	531-025		
Hotstart Taq DNA		500 U	531-050	(2.5 ∪/µℓ)	
polymenase		1,000 l	531-100		
		20 µl	521-200	1 1 2 1	
T D .	04.1	50 µl	521-500	lyophilized	
laq Premix	96 tubes	20 µl	526-200	1.2	
		50 µl	526-500	solution	
		20 µl	522-200		
or T D '	04.1	50 µl	522-500	iyopnilized	
C - laq Premix	96 tubes	20 µl	527-200	1.2	
		50 µl	527-500	solution	
		20 µl	525-200	1.2	
HS-Taq Premix	96 tubes	50 μ l	525-500	solution	
		20 µl	520-200	lyophilized	
Taq Premix (w/o dye)	96 tubes	s 20 µl	524-200	lyophilized	
dNTPs mix		500 µl	509-020	2.5 mM ea	
dNTPs set (set of dATP, dCTP, dGTP and	d dTTP)	I ml x 4 tubes	509-040	100 mM	

* Each dNTPs is available

Products !	Scale	Size	Cat. No.	Тур
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Scale Size Cat. No. Type

GeneAll[®] AmpMasterTM for PCR amplification

Tra Mastar asia	0.5 ml x 2 tubes	541-010	solution
laq Master mix	0.5 ml x 10 tubes	541-050	solution
lpha-Taq Master mix	0.5 ml x 2 tubes	542-010	solution
	0.5 ml x 10 tubes	542-050	solution
LIC Tra Master as	0.5 ml x 2 tubes	545-010	solution
HS-Iaq Master mix	0.5 ml x 10 tubes	545-050	solution

GeneAll[®] HyperScript[™] for Reverse Transcription

Reverse Transcript	ase 10,000 U	601-100	(200 ∪/µℓ)
RT Master mix	$0.5 \text{ ml} imes 2 ext{ tubes}$	601-710	solution
RT Premix	96 tubes, 20 μ ℓ	601-602	solution
Onestep RT-PCR Master mix	$0.5~{\rm ml} imes 2$ tubes	602-110	solution
Onestep RT-PCR Premix	96 tubes, 20 μ ℓ	602-102	solution



GeneAll[®] Exgene[™] Viral DNA/RNA kit Protocol Handbook **I9**



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