Ver 1.2

Handbook for

Viral DNA/RNA

exgene™

DNA 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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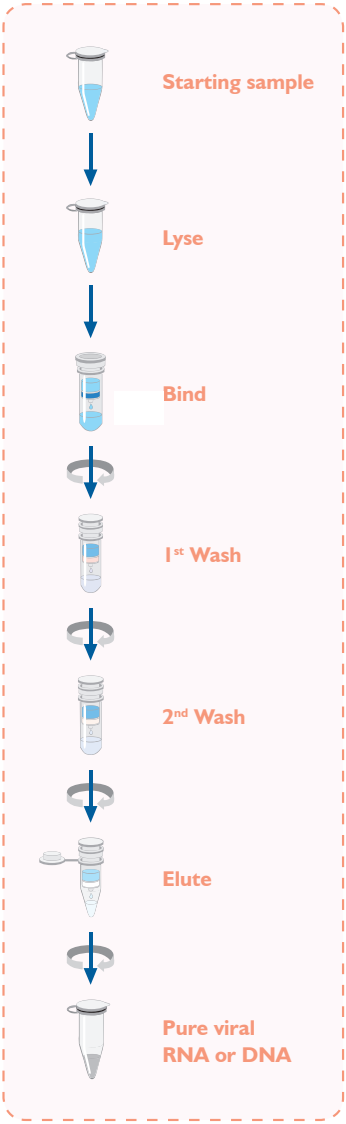
This protocol handbook is included in :

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

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

Brief Protocol



10 μl of Proteinase K solution+200 μl sample

↓

200 μl Buffer BL + 7 μl of Carrier RNA Vortexing
10 sec
Incubation 10 min, 56°C

↓

Lysate + 400 μl Buffer RB1
Vortexing 10 sec
(on mini column)
Centrifugation 1 min, 10,000 x g

↓

500 μl Buffer BW (on mini column)
Centrifugation 1 min, 10,000 x g

↓

700 μl Buffer TW (on mini column)
Centrifugation 1 min, 10,000 x g
Additional centrifugation 1 min, 10,000 x g

↓

20~50 μl Nuclease-free water (on mini column)
Centrifugation 1 min, 10,000 x g

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Kit Contents

Cat. No.	128-150	Storage
Components	Quantity	
Buffer BL	15 ml	Room temperature (15~25°C)
Buffer RB1 (concentrate) *	5 ml	
Buffer BW (concentrate) *	16 ml	
Buffer TW (concentrate) *	10 ml	
Nuclease-free water	15 ml	
Proteinase K **	13 mg	
PK Storage buffer **	1 ml	
Carrier RNA **	370 μg	
Column Type Micro S (with collection tube)	50	
1.5 ml microcentrifuge tube	50	
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** Before first use, add absolute ethanol (ACS grade or better) into Buffer RB1, Buffer BW and Buffer TW as indicated on the bottle.*

*** Refer to instrution of Proteinase K and Carrier RNA on page 8.*

Materials Not Provided

- **Reagents** : Absolute ethanol (ACS grade or better)
- **Disposable materials** : RNase free pipet tips, Disposable gloves
- **Equipment** : Equipment for disrupting sample, Microcentrifuge, Vortex mixer
  Suitable protector (ex; lab coat, goggles, etc)

Product Specifications

Exgene™ Viral DNA/RNA kit	
Type	Spin
Maximum amount of starting samples	200 μl/prep
Preparation time	≥20 min
Maximum loading volume of mini column	750 μl
Minimum elution volume	20 μl

Quality Control

All components in Exgene™ Viral DNA/RNA 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 Exgene™ Viral DNA/RNA should be stored at room temperature (15~25°C). It should be protected from exposure to direct sunlight.

After reconstitution of Proteinase K with the PK Storage buffer, Proteinase K solution should be stored under 4°C or -20°C. Also, dissolved Carrier RNA should be stored at -20°C for conversation of activity.

During shipment or storage under cool ambient condition, a precipitate can be formed in Buffer BL. In such a case, heat the bottle to 37°C to dissolve completely. Using precipitated buffers will lead to poor DNA recovery. Exgene™ Viral DNA/RNA is guaranteed until the expiration date printed on the product box.

Safety Information

The buffers included in the Exgene™ Viral DNA/RNA kit contain irritants which is 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 BL, RB1, and BW contain chaotropic agents, 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.

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 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 1 μg/μl Carrier RNA solution, add 370 μl 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 solution more than 3 times. For one preparation, 7 μl of dissolved Carrier RNA solution is required.

Proteinase K

This kit provides Proteinase K and PK Storage buffer for dissolving Proteinase K. Reconstituted roteinase K serves efficient viral lysis for most sample types. To obtain a 20 mg/ml Proteinase K solution, add 650 μl of PK Storage buffer to the tube of lyophilized Proteinase K, and mix carefully to avoid foaming.

Proteinase K solution 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.

To store for extended periods of time, it is recommended to store under -20°C.

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-culture supernatant, plasma, serum, swab, urine, and virus-infected samples. 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.

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 microcentrifuge tube with deionized water or low ionic strength buffer. The elute should be treated carefully because nucleic acids are very sensitive to contaminants such as nucleases which are often found on general labware and dust.

Purified nucleic acids can be used directly for PCR, qPCR, RT-PCR, or any downstream application without further manipulation.

Exgene™ Viral DNA/RNA kit Protocol

Before experiment

- *Before first use, add absolute ethanol (ACS grade or better) into Buffer RB1, Buffer BW and Buffer TW as indicated on the bottle.*
- *All centrifugation should be carried out at 10,000 x g above (> 12,000 rpm) at room temperature in a microcentrifuge.*
- *Prepare the water bath to 56°C.*
- *Prepare an aliquot of Carrier RNA solution (1 μg/μl) for use on ice (Refer to page 8).*
- *Prepare Proteinase K solution (20 mg/ml) for first use (Refer to page 8).*
- *If a precipitate has formed in Buffer BL, heat to dissolve at 37°C before use.*

1. **Pipet 10 μl of Proteinase K solution (20 mg/ml) into the bottom of a 1.5 ml microcentrifuge tube (not provided).**

2. **Transfer 200 μl of the starting sample to the tube.**
   If the starting sample volume is less than 200 μl, adjust the volume to 200 μl with 1X PBS.
   Starting sample, such as plasma or serum, should be stored at -70°C in aliquots or long term storage. Repeated freezing and thawing of frozen plasma or serum lead to protein precipitation, causing reduced viral titers and subsequently decreased yield of the isolated viral nucleic acid.
   Besides, protein precipitant will cause clogging of mini column.

3. **Add 200 μl of Buffer BL to the tube.**

4. **Add 7 μl of Carrier RNA solution (1 μg/μl) to the tube and mix thoroughly by vortexing for 10 sec.**
   It is essential to mix the sample and Buffer BL thoroughly for good result.
   In case of large sample volume, increase the amount of Buffer BL and Carrier RNA solution proportionally.

5. **Incubate the tube at 56°C for 10 min.**

6. **Spin down briefly to remove any drops from inside of the lid.**

7. **Add 400 μl of Buffer RB1 to the tube and mix thoroughly by vortexing for 10 sec.**
   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.

8. **Transfer the mixture to a Column Type Micro S. Centrifuge at ≥10,000 x g for 1 min at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube (provided).**
   If the sample volume exceeds 750 μl, repeat this step with the remainder of the sample.

9. **Add 500 μl of Buffer BW to the mini column. Centrifuge at ≥10,000 x g for 1 min at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

10. **Add 700 μl of Buffer TW to the mini column. Centrifuge at ≥10,000 x g for 1 min at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

11. **Centrifuge at full speed for 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 carryover of Buffer TW.

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

13. **Centrifuge at ≥10,000 x g for 1 min 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**	Use always fresh or well-stored sample. Too old or improperly stored sample usually results in low yield and poor quality. Repeated freezing and thawing of the sample should be avoided.
	Low concentration of virus in the sample	Use more the starting sample. If the amount of sample is more than 200 μl, concentrate the volume to 200 μl using a microconcentrator.
	Inefficient or insufficient lysis	For proper lysis, the complete mix of sample and Buffer BL is essential.
	Improper elution	Add Nuclease-free water to the center of the mini column membrane and perform incubation for 1 min before centrifugation.
	Precipitation of Buffer BL	Storage at cool ambient temperature may cause precipitation in Buffer BL. For a good result, any precipitate in the buffer should be dissolved by heating the buffer at 37°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 plasticware.
	Incorrect use of Carrier RNA	Add Carrier RNA solution at lysis step. Omission of Carrier RNA solution may lead to low purification efficiency.
	Degradation of Carrier RNA	Carrier RNA solution should be stored at -20°C in aliquots after reconstitution. Do not freeze-thaw the aliquots of Carrier RNA solution more than 3 times.

Facts	Possible Causes	Suggestions
Eluate does not perform well in downstream application	Buffer RB1, BW, or TW was prepared incorrectly	Check that the concentrated Buffer RB1, BW, and TW were diluted with the correct volume of absolute ethanol.
	Residual ethanol from Buffer TW remains in eluate	Care must be taken for eliminating the carryover of Buffer TW before elution step. The membrane of mini column should be kept completely dry via additional centrifugation or air-drying.
	Use of Buffer BW and TW 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 Buffer TW.

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
Stool-Bead DNA mini	mini	50	115-151	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 without spin column*				
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
Ribospin™ Pathogen/TNA	mini	50	314-150	spin
		250	314-152	
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	
Taq Premix	20 μl x 96 tubes		526-200	solution
	50 μl x 96 tubes		526-500	
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
GeneAll® *HyperScript™* *for Reverse Transcription*				
Reverse Transcriptase		10,000 U	601-100	solution
RT Master mix	0.5 ml x 2 tubes		601-710	solution
One-step RT-PCR Master mix	0.5 ml x 2 tubes		602-110	solution
One-step RT-PCR Premix	20 μl x 96 tubes		602-102	solution
GeneAll® RealAmp™ *for qPCR amplification*				
SYBR qPCR Master mix (2X, Low ROX)	200 rxn	2 ml	801-020	solution
	500 rxn	5 ml	801-050	
SYBR qPCR Master mix (2X, High ROX)	200 rxn	2 ml	801-021	solution
	500 rxn	5 ml	801-051	
GeneAll® Protein series				
ProtinEx™ Animal cell/tissue		100 ml	701-001	solution
PAGESTA™ Reducing 5X SDS-PAGE Sample Buffer	1 ml x 10 tubes		751-001	solution

Products	Size	Cat. No.	Type
GeneAll® GENTi™ 32 ADVANCED *Newly designed automated extraction system*			
Automatic extraction equipment		GTI032A	system
Genomic DNA	48	901-048A	tube
	96	901-096A	plate
Viral DNA/RNA	48	902-048A	tube
	96	902-096A	plate
Blood DNA	48	903-048A	tube
	96	903-096A	plate
Plant DNA/RNA	48	904-048A	tube
	96	904-096A	plate
LMO	48	906-048A	tube
	96	906-096A	plate
Fecal DNA/RNA	48	913-048A	tube
	96	913-096A	plate
GeneAll® AllEx®64 *Compact yet Comprehensive automated extraction system*			
Automatic extraction equipment		AEX064	system
Genomic DNA	48	931-048A	tube
	96	931-096A	plate
Viral DNA/RNA	48	934-048A	tube
	96	934-096A	plate
Blood DNA	48	935-048A	tube
	96	935-096A	plate
Plant DNA/RNA	48	937-048A	tube
	96	937-096A	plate
Fecal DNA/RNA	48	948-048A	tube
	96	948-096A	plate

Note

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GeneAll

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