Ver 1.1

HB1080

Handbook for

■ Stool DNA mini kit

exgene tm

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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Visit GeneAll® Community

www.geneall.com

www.geneall.co.kr

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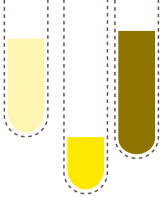
This protocol handbook is included in :

GeneAll® Exgene™ Stool DNA mini (115-150)

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

Brief protocol



Stool sample

Homogenization

1. Add 1 ml Buffer PBS to 200 mg stool samples
2. Homogenize the sample
3. Incubate at RT for 30 sec
4. Transfer the supernatant to a new microcentrifuge tube
5. Centrifuge at full speed for 2 min and discard the supernatant

Lyse

6. Add 1.3 ml Buffer FL and suspend the pellet
7. Incubate at RT for 5 min
8. Centrifuge at ≥ 10,000 x g for 5 min

EzPass™ Filter

9. Transfer the supernatant to a EzPass™ Filter
10. Centrifuge at ≥ 10,000 x g for 1 min
11. Transfer the EzPass™ Filter to a new microcentrifuge tube
12. Repeat step 9~10 using the remainder
13. Add 100 μl Buffer EB into EzPass™ Filter and incubate at RT for 1 min
14. Centrifuge at ≥ 10,000 x g for 1 min

2nd Bind

15. Add 500 μl Buffer PB to the passed-through
16. Transfer the mixture to a mini column
17. Centrifuge at ≥ 10,000 x g for 1 min

Wash

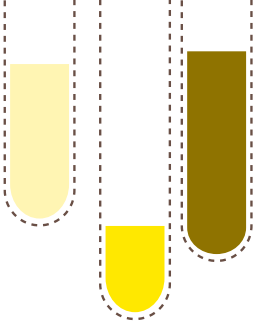
18. Add 500 μl Buffer NW into mini column
19. Centrifuge at ≥ 10,000 x g for 1 min
20. Additional centrifuge at full speed for 1 min

Elute

21. Apply 50 μl Buffer EB into mini column
22. Incubate at RT for 1 min
23. Centrifuge at ≥ 10,000 x g for 1 min

Pure genomic DNA

Pure DNA



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KIT CONTENTS

Cat. No.	115-150	
Components	**Quantity**	**Storage**
No. of preparation	50	Room temperature (15~25°C)
Buffer PBS	60 ml	
Buffer FL	70 ml	
Buffer EB **	15 ml	
Buffer PB	30 ml	
Buffer NW (concentrate) * †	6 ml	
EzPass™ Filter (with collection tube)	50	
Column Type G (mini) (with collection tube)	50	
1.5 ml microcentrifuge tube	100	
2.0 ml microcentrifuge tube	100	
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** Before using for the first time, add absolute ethanol (ACS grade or better) into Buffer NW as indicated on the bottle.*

† Contains sodium azide as a preservative

*** 10 mM TrisCl, pH 8.5*

Materials Not Provided

Disposable material : Pipette tips, Disposable gloves

Equipment : Microcentrifuge, Vortex mixer, Suitable protector (ex; lab coat, goggles, etc.)

Product Specifications

Exgene™ Stool DNA mini	
Type	Spin
Maximum amount of starting samples	200 mg/prep
Preparation time	≥25 min
Maximum loading volume of mini column	750 μl
Minimum elution volume	30 μl
Maximum binding capacity	100 μg

Quality Control

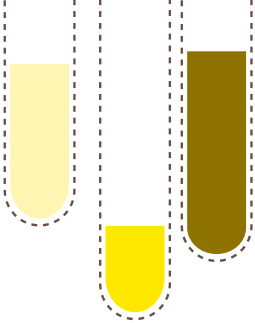
All components in GeneAll® Exgene™ Stool DNA mini kit are manufactured in strictly clean conditions, and its degree of cleanness is monitored periodically. Quality control is carried out thoroughly from lot to lot, and only the qualified kits are approved to be delivered.

Storage Conditions

All components of GeneAll® Exgene™ Stool DNA mini kit should be stored at room temperature (15~25°C). It should be protected from exposure to direct sunlight. During shipment or storage under cool ambient condition, a precipitate can be formed in Buffer FL and PB. In such a case, heat the bottle to 50°C to dissolve completely. Using precipitated buffers will lead to poor DNA recovery. GeneAll® Exgene™ Stool DNA mini kit is guaranteed until the expiration date printed on the product box.

Safety Information

The buffers included in the GeneAll® Exgene™ Stool DNA mini 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 FL and PB 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.



Product Description

GeneAll Exgene™ Stool DNA mini kit provides a convenient method for the isolation of total DNA from stool samples. This kit utilizes a double binding procedure using the optimized buffer system and the advanced silica binding technology to purify nucleic acid suitable for many applications. Through this method, the contained impurities in the starting stool samples are removed so that high quality DNA can be purified from host and microbial cells. The stool samples can be applied up to 200 mg per prep and this procedure can be completed in 25 minutes.

This procedure is started with homogenization and lysis steps. The lysate is applied to EzPass™ Filter and then the stool DNA is eluted by centrifugation, the first binding step.

After the first elution, the eluate is mixed with DNA binding buffer and the stool DNA is bound on the silica membrane. Following washing step, the bound DNA is eluted by elution buffer, the second elution. Purified DNA can be directly applicable in conventional PCR, restriction analysis, electrophoresis, and any other downstream applications.

Exgene™
Stool DNA mini protocol

1. **Add up to 200 mg of stool sample to a 2 ml microcentrifuge tube (provided).**

2. **Add 1 ml of Buffer PBS to the tube and vortex for 1 min or until the stool sample is thoroughly homogenized.**
   In case of bird droppings, use 1.6 ml of Buffer PBS.
   It is important to homogenize the sample thoroughly. Insufficient homogenization time and condition is related to low recovery yield.
   To help the homogenization, crush the sample using a wide-bore tip or cut the end off the pipet tip before vortexing.

3. **Stand the tube for 30 sec at room temperature.**

4. **Transfer the supernatant to a new 2 ml microcentrifuge tube.**
   It may requisite to use a wide-bore tip or cut the end off the pipet tip to apply the viscous homogenate to the tube.

5. **Centrifuge the tube at full speed for 2 min and discard the supernatant.**

6. **Add 1.3 ml of Buffer FL and resuspend the pellet by pipetting up and down.**
   To enhance the resuspension, vortex the tube after pipetting can be helpful. If Buffer FL precipitation, pre-heat in a 56°C water bath to dissolve completely.

7. **Stand the tube at room temperature for 5 min and then centrifuge at ≥10,000 x g for 5 min at room temperature.**
   If possible, move the supernatant to a new 1.5 ml microcentrifuge tube before step 8.

8. Transfer the supernatant to a EzPass™ Filter (white).

9. Centrifuge at ≥10,000 x g for 1 min at room temperature.

10. Repeat step 8~9 using the remainder of the sample.
    Transfer the EzPass™ Filter to a new 1.5 ml microcentrifuge tube (provided).

11. Add 100 μl of Buffer EB to the EzPass™ Filter and incubate for 1 min at room temperature.

12. Centrifuge at ≥10,000 x g for 1 min at room temperature.

13. Add 500 μl of Buffer PB to the passed-through and mix well by pipetting.

14. Transfer the mixture to a Column Type G (green).

15. 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.

16. Add 500 μl of Buffer NW to the mini column.

17. 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.

18. **Centrifuge at maximum speed for an additional 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 NW.

19. **Add 50 μl of Buffer EB to the center of the membrane in the mini column Incubate for 1 min at room temperature.**
    Centrifuge at ≥10,000 x g for 1 min at room temperature.
    Elution volume can be decreased to 30 μl for high concentration of DNA, but this will slightly decrease in overall DNA yield. If maximum recovery of DNA is prefered or the starting materials contain large amount of DNA, elution can be done in 200 μl of Buffer EB.

Trouble shooting

Facts	Possible Causes	Suggestions
Low or no recovery	Incorrect sample storage	Sample should be stored at 4°C or -20°C.
	Too much starting material	Too much starting material lead to inefficient homogenization, followed by poor DNA yields. Reduce the amount of starting material down to 200 mg per prep.
	Insufficient Homogenization	Check the step 2 of protocol. Insufficient homogenization time and condition is related to low recovery yield.
	Incomplete lysis	Check the step 6 of protocol. Incomplete lysis process leads to low recovery yield. Be sure to mix the pellet in correct volume of Buffer FL by pipetting.
Column clogging	Incomplete Homogenization	Be sure to mix the pellet in correct volume of Buffer FL by pipetting. And centrifuge again until the lysate has passed through the membrane.
	Too much starting sample	Too much starting sample can lead to column clogging. Reduce the amount of starting material down to 200 mg per prep.
Low efficiency of DNA amplification	Excess amount of template DNA	An excess amount of template DNA will inhibit a PCR reaction. The template DNA is needed to dilute.
Eluate does not preform well in the downstream application	Residual ethanol remains in eluate	To remove any residual ethanol included in Buffer NW from the mini column membrane, centrifuge again for complete removal of ethanol.

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
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 x 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

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GeneAll

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