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Handbook for

■ Stool DNA mini kit

exgene tm

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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This protocol handbook is included in :

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

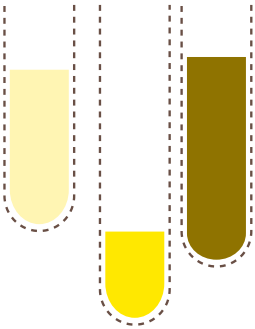
Visit www.geneall.com or www.geneall.co.kr for FAQ, QnA and more information.

Brief protocol

GeneAll

GENEALL BIOTECHNOLOGY CO., LTD

Sample homogenization step	Add up to 200mg of stool sample to a 2 ml tube. Add 1 ml of Buffer PBS. Homogenize the sample. Stand the tube for 30 sec. Transfer the supernatant to a new 2 ml tube. Centrifuge the tube at full speed for 2 min.
Sample lysis step	Add 1.3 ml of buffer FL and pipetting well. Stand the tube at room temperature for 5 min. Centrifuge at ≥ 10,000 x g for 5 min.
The first binding step	Transfer the supernatant to a EzPass™ filter. Centrifuge at ≥ 10,000 x g for 1 min. Add 100 ul of buffer EB to the EzPass™ filter. Centrifuge at ≥ 10,000 x g for 1 min.
The second binding step	Add 500 ul of buffer PB to the passed-through. Transfer the mixture to a mini spin column. Centrifuge at ≥ 10,000 x g for 1 min.
Washing step	Add 500 ul of buffer NW to the mini spin column. Centrifuge at ≥ 10,000 x g for 1 min. Centrifuge at maximum speed for 1 min.
DNA elution step	Add 50 ul of buffer EB to the membrane. Centrifuge at ≥ 10,000 x g for 1 min.



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KIT CONTENTS

GeneAll® Exgene™ Stool DNA mini

Cat. No	115-150	
Components	**Quantity**	**Storage**
Buffer PBS	60 ml	Room temperature (15 ~ 25°C)
Buffer FL	70 ml	
Buffer EB	15 ml	
Buffer PB	30 ml	
Buffer NW	30 ml	
EzPass filter (with collection tube)	50	
GeneAll Column type G (with collection tube)	50	
1.5 ml tube	100	
2.0 ml tube	100	

Product Specifications

Specification	Stool DNA mini
Type	Spin
Maximum amount of starting samples	~ 200 mg stool sample
Maximum loading volume of spin column	~ 700 ul
Minimum elution volume	~ 30 ul
Maximum binding capacity	~ 100 ug

Quality Control

GeneAll® Exgene™ Stool DNA mini kit is 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 lot is approved to be delivered.

Storage Conditions

GeneAll® Exgene™ Stool DNA mini kit should be stored at room temperature (15 ~ 25°C). In cold ambient condition, buffer FL and PB may exhibit salt precipitation and this will cause reduction of DNA recover-yields. If so, heat the bottle with occasional swirling in 37°C water bath until completely dissolved.
All components are stable for 1 year.

Precautions

The buffers included in GeneAll® Exgene™ Stool DNA mini kit 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. In case of contact, wash immediately with plenty of water and seek medical advice.
Buffer FL and PB contain chaotropes. It can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste.

Product Disclaimer

GeneAll® Exgene™ Stool DNA mini kit is for research use only, not for use in diagnostic procedure.

Materials Not Provided

Disposable material

- Pipet tips
- Disposable gloves

Equipment

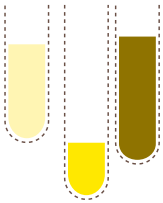
- Microcentrifuge
- Suitable protector (ex; lab coat, disposable gloves, goggles, etc)

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, rescriction analysis, electrophoresis, and any other downstream applications.



Exgene™
Stool DNA mini protocol

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

2. **Add 1 ml of Buffer PBS to the tube and vortex for 1 minute 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 seconds at room temperature.**

4. **Transfer the supernatant to a new 2 ml tube (provided).**
   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 minutes 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 minutes and then centrifuge at ≥ 10,000 x g for 5 minutes at room temperature.**
   If possible, move the supernatant to a new 1.5 ml tube before step 8.

8. **Transfer the supernatant to a EzPass™ filter (white column).**

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

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

11. **Add 100 ul of buffer EB to the EzPass filter and incubate for 1 minute at room temperature.**

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

13. **Add 500 ul of buffer PB to the passed-through and mix well by pipetting.**

14. **Transfer the mixture to a mini spin column (G type, green).**

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

16. **Add 500 ul of buffer NW to the mini spin column.**

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

18. **Centrifuge at maximum speed for 1 minute at room temperature to remove residual wash buffer.**
Transfer the mini spin column to a new 1.5 ml 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 ul of buffer EB to the center of the membrane in the mini spin column.**
Incubate for 1 minute at room temperature. Centrifuge at ≥ 10,000 x g for 1 minute at room temperature.

Elution volume can be decreased to 30 ul 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 ul 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	Sample not homogenized completely	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 amonut 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 mini spin 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		50	100-150	mini / spin
		100	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	mini	50	114-150	spin
Stool	mini	50	115-150	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	228-201	
	Lx	20	228-301	
GeneAll® DirEx™ *for preperation of PCR-template without extraction*				
DirEx™		50	250-050	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
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	

Products	Scale	Size	Cat. No.	Type
GeneAll® *AmpONE™* *for PCR amplification*				
Hotstart Taq DNA polymerase		250 U	531-025	(2.5 U/㎕)
		500 U	531-050	
		1,000 U	531-100	
Clean Taq DNA polymerase		250 U	551-025	(2.5 U/㎕)
		500 U	551-050	
		1,000 U	551-100	
Clean α-Taq DNA polymerase		250 U	552-025	(2.5 U/㎕)
		500 U	552-050	
		1,000 U	552-100	
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	
Taq Premix (w/o dye)	96 tubes	20 ㎕	524-200	lyophilized
α-Taq Premix (w/o dye)	96 tubes	20 ㎕	525-200	solution
dNTP mix		500 ㎕	509-020	2.5 mM each
dNTP set (set of dATP, dCTP, dGTP and dTTP)		1 ml x 4 tubes	509-040	100 mM
GeneAll® *AmpMaster™* *for PCR amplification*				
Taq Master mix		2x	511-010	0.5 ml x 2 tubes
		2x	511-050	0.5 ml x 10 tubes
α-Taq Master mix		2x	512-010	0.5 ml x 2 tubes
		2x	512-050	0.5 ml x 10 tubes
HS-Taq Master mix		2x	545-010	0.5 ml x 2 tubes
		2x	545-050	0.5 ml x 10 tubes

* Each dNTP is available

GeneAll

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