Ver 2.0

HB1150

Handbook for

Soil DNA mini

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

This protocol handbook is included in :

GeneAll® Exgene™ Soil DNA mini (114-150)

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

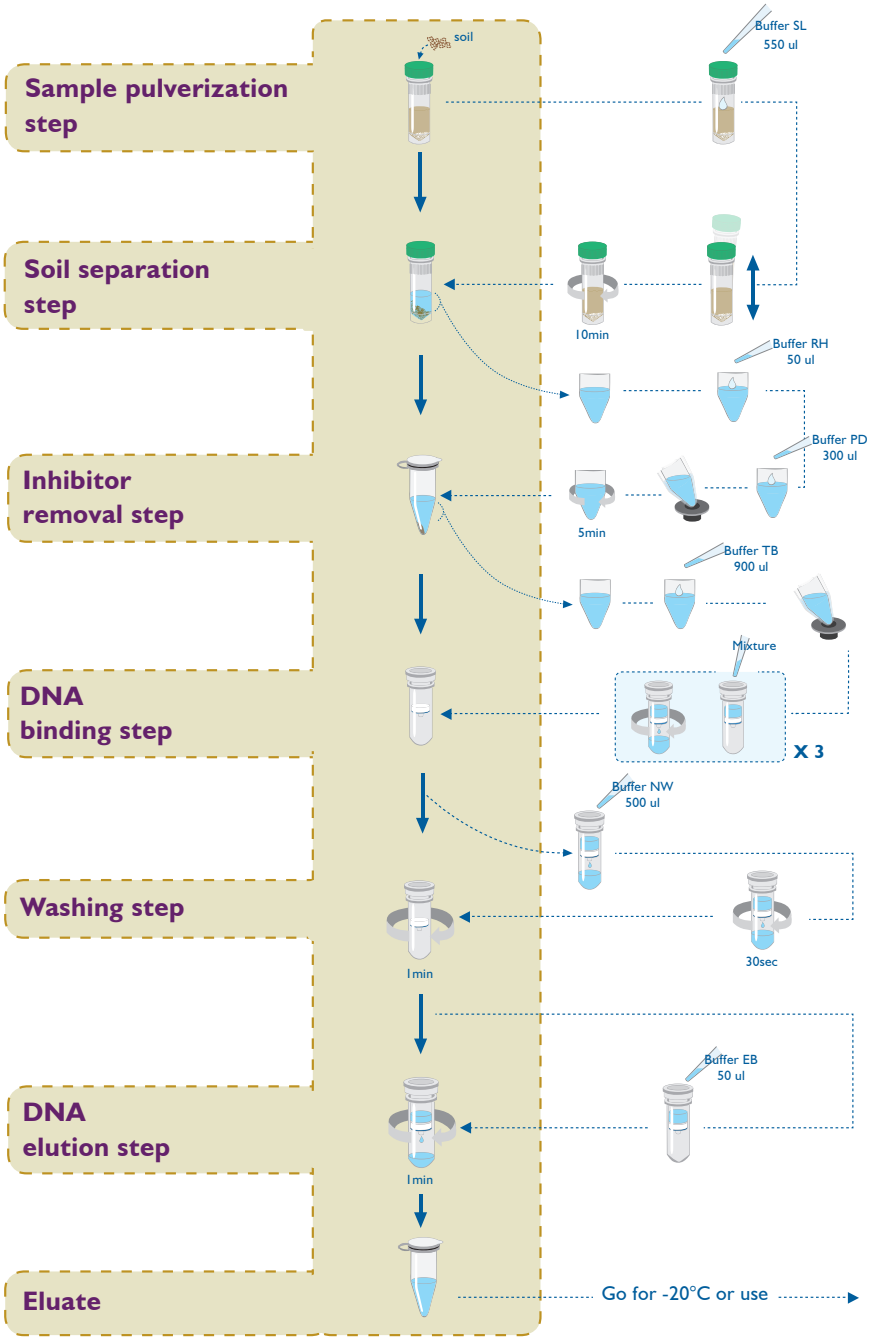
Brief Protocol

GeneAll

GENEALL BIOTECHNOLOGY CO., LTD

Sample pulverization step	Add up to 500 mg of soil sample to a Powerbead™ tube. Add 550 ul of buffer SL. Pulverize the sample. Centrifuge at ≥ 10,000 x g for 10 minutes.
Inhibitor removal step	Transfer the supernatant to a 1.5 ml microcentrifuge tube. Add 50 ul of buffer RH. Add 300 ul of buffer PD and mix well. Centrifuge at ≥ 10,000 x g for 5 minutes.
DNA binding step	Transfer the supernatant to a 2.0 ml microcentrifuge tube. Add 900 ul of buffer TB. Apply the mixture into a mini spin column and centrifuge at ≥ 10,000 x g for 30 seconds.
Washing step	Add 500 ul of buffer NW and Centrifuge at ≥ 10,000 x g for 30 seconds. Centrifuge at ≥ 10,000 x g for 1 minute.
DNA elution	Add ~50 ul of buffer EB to the center of the membrane. Centrifuge at ≥ 10,000 x g for 1 minute.

Brief Protocol



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KIT CONTENTS

Components	Quantity	Storage
Buffer SL	30 ml	
Buffer RH	3 ml	
Buffer PD	17 ml	
Buffer TB	50 ml	
Buffer NW (concentrate) * †	6 ml	Room temperature
Buffer EB	15 ml	
Powerbead™ tube	50	
GeneAll® Column type G (with collection tube)	50	
1.5 ml microcentrifuge tube	100	
2.0 ml microcentrifuge tube	50	

** 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.*

MATERIALS NOT PROVIDED

Reagent

- Absolute ethanol, ACS grade or better

Disposable material

- Pipet tips
- Disposable gloves

Equipment

- Precellys® 24 (Bertin, France) equipment or any equivalent
- Microcentrifuge
- Suitable protector (ex; lab coat, disposable gloves, goggles, etc)

QUALITY CONTROL

GeneAll® Exgene™ Soil DNA mini 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 is approved to be delivered.

STORAGE CONDITIONS

GeneAll® Exgene™ Soil DNA mini should be stored at room temperature (15~25°C). But prolonged storage at high temperature over 30°C can reduce the performance of the kit.

In cold ambient condition, buffer RH and TB 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.

Keep out of direct sunlight.

PRECAUTIONS

The buffers included in GeneAll® Exgene™ Soil DNA mini 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 TB contains 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™ Soil DNA mini is for research use only, not for use in diagnostic procedure.

Product Specifications

Specification	Exgene™ Soil DNA mini
Type	Spin
Maximum amount of starting samples	500 mg soil sample
Maximum loading volume of spin column	700 ul
Minimum elution volume	30 ul
Maximum binding capacity	100 ug

Product Description

GeneAll® Exgene™ Soil DNA mini provides a convenient method for the isolation of total DNA from soil samples. This kit utilizes the powerful beads, the optimized buffer system and the advanced silica binding technology to purify nucleic acid suitable for many applications. These complex systems of this kit can deal with a number of different types of samples in the soil including plant tissues, bacteria, fungi spores and others. Also, it removes a humic acid and other PCR inhibitors from various soil samples efficiently. The humic acid, which is a sort of brownish colour, is a critical factor for soil treating experiments and if remained in eluate, this can have a negative effect on the DNA downstream applications.

GeneAll® Exgene™ Soil DNA mini provides a tube including powerful beads for strong pulverization. Soil samples are placed in this tube with lysis buffer, buffer SL, and crushed by bead-beater or vortex. After centrifugation, supernatant is mixed with precipitation buffer, buffer RH and buffer PD, to precipitate humic acid and protein. Then, the separated DNA part, supernatant, blend into the binding buffer, buffer TB, and DNA is bound on the silica membrane through centrifugation. Following washing step with buffer NW, the bound DNA is eluted by buffer EB. Purified DNA can be directly applicable in conventional PCR, restriction analysis, electrophoresis, and any other downstream applications.

PROTOCOL FOR

Exgene™ Soil DNA mini

1. **Add up to 500 mg of soil sample to a Powerbead™ tube.**

2. **Add 550 ul of buffer SL to the tube.**

3. **Homogenize the sample in the Precellys® 24 (Bertin, France) equipment for twice of 23 seconds at 6500 rpm.**
   Alternatively, secure tubes horizontally on a flat-bed vortex pad with tape and vortex at maximum speed for 10 minutes.

4. **Centrifuge at ≥10,000 x g for 10 minutes at room temperature and carefully transfer the supernatant to a 1.5 ml microcentrifuge tube (provided).**

5. **Add 50 ul of buffer RH.**

6. **Add 300 ul of buffer PD and mix well by vortexing.**

7. **Centrifuge at ≥10,000 x g for 5 minutes at room temperature and carefully transfer the supernatant to a 2.0 ml microcentrifuge tube (provided).**
   Small pellet containing humic acid, cell debris, and protein can be formed in the collection tube after centrifugation. Be careful not to disturb this pellet.

8. **Add 900 ul of buffer TB and mix well by vortexing.**
   If buffer TB precipitation, pre-heat in a 56°C water bath to dissolve completely.

9. **Transfer up to 700 ul of the mixture to a mini spin column.**

10. **Centrifuge at ≥10,000 x g for 30 seconds at room temperature.**
    Discard the pass-through and reinsert the mini spin column back into the same tube.

11. Repeat two more times step 9~10 using the remainder of the sample.

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

13. Centrifuge at ≥10,000 x g for 30 seconds at room temperature.
Discard the pass-through and reinsert the mini spin column back into the same tube.

14. 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 microcentrifuge tube (provided).
Residual ethanol may interfere with downstream reactions. Care must be taken at this step for eliminating the carryover of buffer NW.

15. 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.

Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low or no recovery	**Too much starting material**	Too much starting material lead to inefficient lysis, followed by poor DNA yields. Reduce the amount of starting material.
	Insufficient Homogenization	Check the step 3 of protocol. Insufficient homogenization time and condition is related to low recovery yield.
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 perform 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.
DNA eluate is brown	**Humic acid is not be removed completely**	With certain samples, a little humic acid can be remained in the eluate. In this case, we recommend using a GeneAll® Expin™ CleanUp SV kit to purify contaminated eluate.

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/㎕)
		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	
α-Pfu DNA polymerase		250 U	504-025	(2.5 U/㎕)
		500 U	504-050	
		1,000 U	504-100	
Fast-Pfu DNA polymerase		250 U	505-025	(2.5 U/㎕)
		500 U	505-050	
		1,000 U	505-100	
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
α-Pfu Premix	96 tubes	50 ㎕	523-500	solution
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

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
PAGESTA™ Reducing 5X SDS-PAGE Sample Buffer	1 ml × 10 tubes	751-001
GeneAll® STEADi™ *for automatic nucleic acid purification*		
STEADi™ 12 Instrument		GST012
STEADi™ 24 Instrument		GST024
STEADi™ Genomic DNA Cell / Tissue Kit	96	401-104
STEADi™ Genomic DNA Blood Kit	96	402-105
STEADi™ Bacteria DNA Kit	96	403-106
STEADi™ Total RNA Kit	96	404-304
STEADi™ Viral DNA / RNA Kit	96	405-322
STEADi™ CFC Seed DNA / RNA Kit	96	406-C02

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