Ver 1.0

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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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

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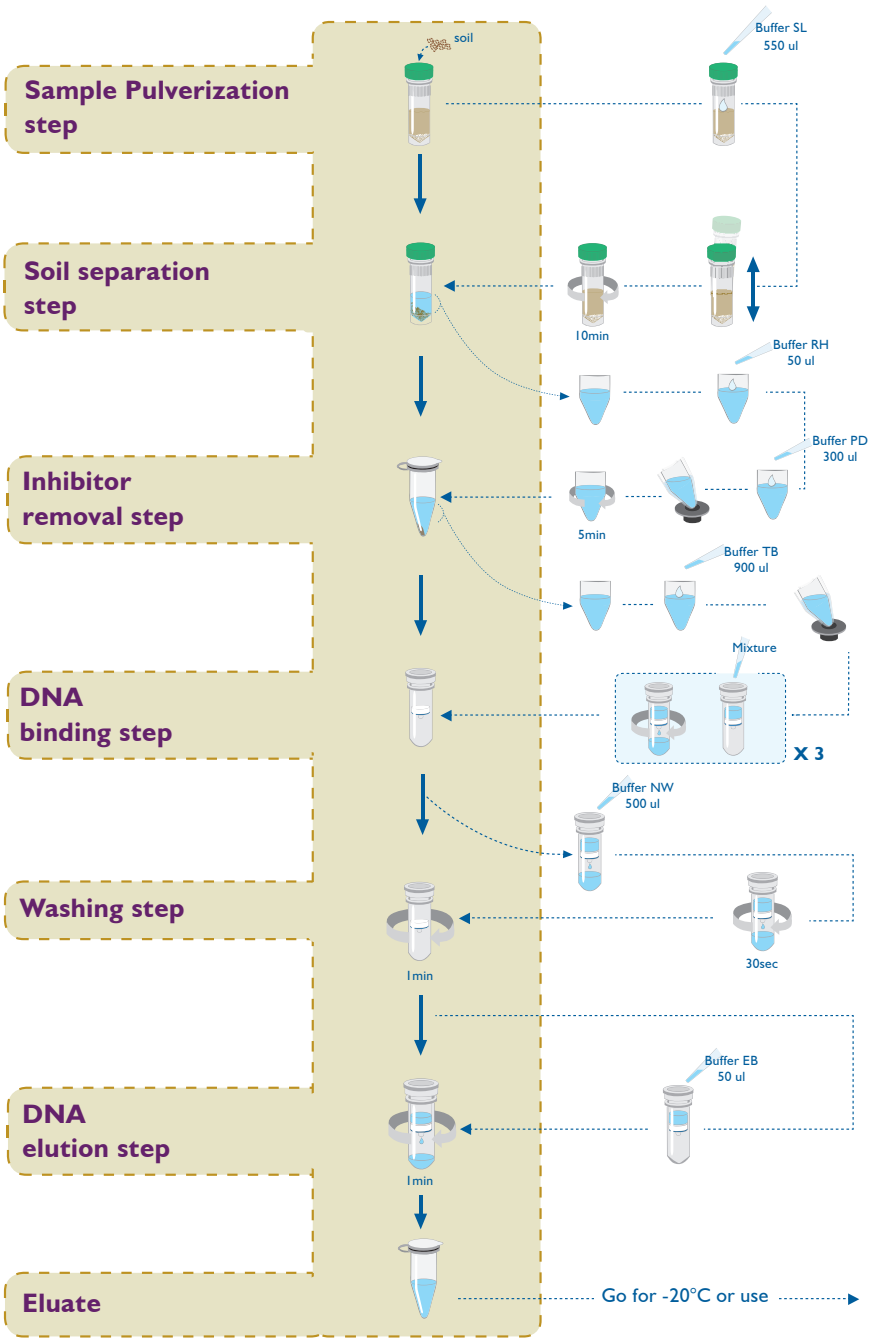
GENEALL BIOTECHNOLOGY CO., LTD

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 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 ml 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	Room temperature
Buffer RH	3 ml	
Buffer PD	17 ml	
Buffer TB	50 ml	
Buffer NW	30 ml	
Buffer EB	15 ml	
Powerbead™ tube	50	
GeneAll® Column type G (with collection tube)	50	
1.5 ml tube	100	
2.0 ml tube	50	

MATERIALS NOT PROVIDED

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 provide 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 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 ml 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 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 for Exgene™ Soil DNA mini

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 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.
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 Clean up SV kit to purify contaminated eluate.

Ordering Information

Products	Type	Size	Cat. No.
GeneAll® *Hybrid-Q™* *for rapid preparation of plasmid DNA*			
Plasmid Rapidprep	mini / spin	50	100-150
		200	100-102
GeneAll® *Exprep™* *for preparation of plasmid DNA*			
Plasmid SV mini	spin / vacuum	50	101-150
		200	101-102
		1,000	101-111
Plasmid SV Midi**	spin / vacuum	26	101-226
		50	101-250
		100	101-201
GeneAll® *Exfection™* *for preparation of highly pure plasmid DNA*			
Plasmid LE mini *(Low Endotoxin)*	spin / vacuum	50	111-150
		200	111-102
Plasmid LE Midi* *(Low Endotoxin)*	spin / vacuum	26	111-226
		100	111-201
Plasmid EF Midi* *(Endotoxin Free)*	spin	20	121-220
		100	121-201
GeneAll® *Expin™* *for purification of fragment DNA*			
Gel SV	mini / spin / vacuum	50	102-150
		200	102-102
PCR SV	mini / spin / vacuum	50	103-150
		200	103-102
CleanUp SV	mini / spin / vacuum	50	113-150
		200	113-102
Combo GP	mini / spin / vacuum	50	112-150
		200	112-102
GeneAll® *Exgene™* *for isolation of total DNA*			
Tissue SV mini*	spin / vacuum	100	104-101
		250	104-152
Tissue SV Midi**	spin / vacuum	26	104-226
		100	104-201
Tissue SV MAXI**	spin / vacuum	10	104-310
		26	104-326
Tissue *plus!* SV mini*	spin / vacuum	100	109-101
		250	109-152
Tissue *plus!* SV Midi**	spin / vacuum	26	109-226
		100	109-201
Tissue *plus!* SV MAXI**	spin / vacuum	10	109-310
		26	109-326

Products	Type	Size	Cat. No.
GeneAll® *Exgene™* *for isolation of total DNA*			
Blood SV mini	spin / vacuum	100	105-101
		250	105-152
Blood SV Midi**	spin / vacuum	26	105-226
		100	105-201
Blood SV MAXI**	spin / vacuum	10	105-310
		26	105-326
Cell SV mini	spin / vacuum	100	106-101
		250	106-152
Cell SV MAXI**	spin / vacuum	10	106-310
		26	106-326
Clinic SV mini	spin / vacuum	100	108-101
		250	108-152
Clinic SV Midi	spin / vacuum	26	108-226
		100	108-201
Clinic SV MAXI**	spin / vacuum	10	108-310
		26	108-326
Genomic DNA micro	spin	50	118-050
Plant SV mini	spin / vacuum	100	117-101
		250	117-152
Plant SV Midi**	spin / vacuum	26	117-226
		100	117-201
Plant SV MAXI**	spin / vacuum	10	117-310
		26	117-326
GMO SV mini	spin / vacuum	50	107-150
		200	107-102
Soil mini	spin	50	114-150
GeneAll® *GenEx™* *for isolation of total DNA*			
GenEx™ B	Sx† / solution	100	220-101
	Sx† / solution	500	220-105
	Lx†† / solution	100	220-301
GenEx™ C	Sx† / solution	100	221-101
	Sx† / solution	500	221-105
	Lx†† / solution	100	221-301
GenEx™ T	Sx† / solution	100	222-101
	Sx† / solution	500	222-105
	Lx†† / solution	100	222-301
GeneAll® *DirEx™* *Single tube DNA extraction buffer for PCR*			
DirEx™	solution	50	250-050

Products	Type	Size	Cat. No.
GeneAll® *RNA Series* *for preparation of RNA*			
RiboEx™	solution	100	301-001
		200	301-002
Hybrid-R™	spin	100	305-101
Hybrid-R™ Blood RNA	spin	50	315-150
Hybrid-R™ miRNA	spin	50	325-150
RiboEx™ LS	solution	100	302-001
		200	302-002
Riboclear™	spin	50	303-150
Ribospin™	spin	50	304-150
Ribospin™ vRD	spin	50	302-150
Ribospin™ Plant	spin	50	307-150
Allspin™	spin	50	306-150
GeneAll® *AmpONE™* *for PCR amplification*			
Taq DNA polymerase	(2.5 U/μℓ)	250 U	501-025
		500 U	501-050
		1,000 U	501-100
α-Taq DNA polymerase	(2.5 U/μℓ)	250 U	502-025
		500 U	502-050
		1,000 U	502-100
Pfu DNA polymerase	(2.5 U/μℓ)	250 U	503-025
		500 U	503-050
		1,000 U	503-100
Hotstart Taq DNA polymerase	(2.5 U/μℓ)	250 U	531-025
		500 U	531-050
		1,000 U	531-100
Clean Taq DNA polymerase	(2.5 U/μℓ)	250 U	551-025
		500 U	551-050
		1,000 U	551-100
Clean α-Taq DNA polymerase	(2.5 U/μℓ)	250 U	552-025
		500 U	552-050
		1,000 U	552-100
Taq Master mix	0.5 ml x 2 tubes	2x	511-010
	0.5 ml x 10 tubes	2x	511-050
α-Taq Master mix	0.5 ml x 2 tubes	2x	512-010
	0.5 ml x 10 tubes	2x	512-050

Products	Type	Size	Cat. No.
GeneAll® *AmpONE™* *for PCR amplification*			
Taq Premix	96 tubes	20 μℓ	521-200
		50 μℓ	521-500
α-Taq Premix	96 tubes	20 μℓ	522-200
		50 μℓ	522-500
Taq Premix (w/o dye)	96 tubes	20 μℓ	524-200
α-Taq Premix (w/o dye)	96 tubes	20 μℓ	525-200
dNTP mix	2.5 mM each	500 μℓ	509-020
dNTP set (set of dATP, dCTP, dGTP and dTTP)	100 mM	1 ml x 4 tubes	509-040

* Each dNTP is available

** GeneAll® Tissue SV mini, Midi, and MAXI plus! kit provide the additional methods for the purification from animal whole blood.*
*** GeneAll® SV Midi / MAXI kits require the centrifuge which has a swinging-bucket rotor and ability of 4,000 ~ 5,000 xg.*
† On the basis of DNA purification from 300 ul whole blood, 2 x 10^6 cells or 10 mg animal tissue.
†† On the basis of DNA purification from 10 ml whole blood. 1 x 10^8 cells or 100 mg animal tissue.

GeneAll

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