Ver 3.0

HB1150

Handbook for

Soil DNA mini

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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www.geneall.com

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This protocol handbook is included in :

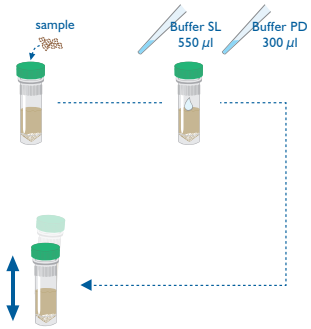
GeneAll® Exgene™ Soil DNA mini (114-150)

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

INDEX

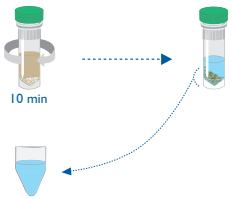
Brief Protocol

Homogenization step



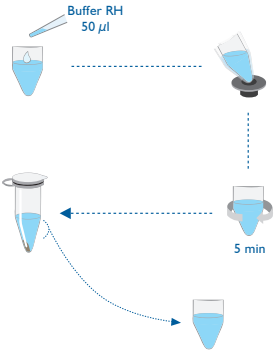
1. Add sample to a Power bead.
2. Add 550 µl of Buffer SL.
3. Add 300 µl of Buffer PD.
4. Homogenize the sample.

Separation step



5. Centrifuge at ≥ 10000 xg for 10 min and transfer the supernatant to a 1.5 ml microcentrifuge tube.

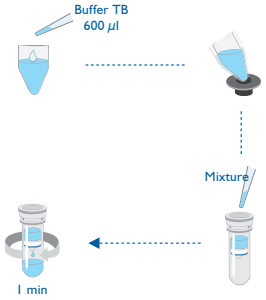
Inhibitor removal step



6. Add 50 µl of Buffer RH and mix well.
7. Centrifuge at ≥ 10000 xg for 5 min and transfer the supernatant to a 1.5 ml microcentrifuge tube.

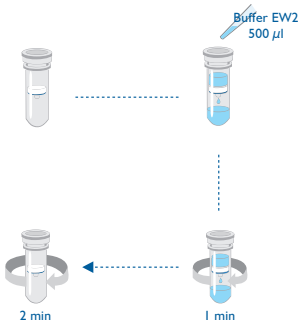
Brief Protocol

DNA binding step



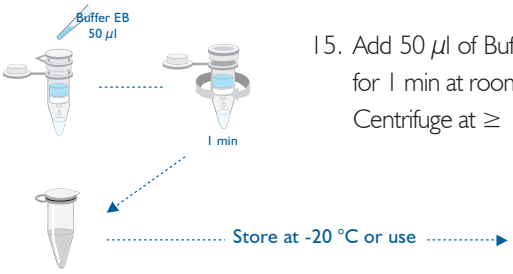
8. Add 600 µl of Buffer TB and mix well.
9. Apply the mixture into a mini spin column.
10. Centrifuge at ≥ 10000 xg for 1 min.
11. Repeat 9-10

Washing step



12. Add 500 µl of Buffer EW2.
13. Centrifuge at ≥ 10000 xg for 1 min.
14. Centrifuge at full speed for 2 min.

DNA elution step



15. Add 50 µl of Buffer EB and incubate for 1 min at room temperature. Centrifuge at ≥ 10000 xg for 1 min.

KIT CONTENTS

Components	Quantity	Storage
Buffer SL	30 ml	Room temperature (15 °C to 25 °C)
Buffer RH	3 ml	
Buffer PD	17 ml	
Buffer TB	35 ml	
Buffer EW2 (concentrate) *	6 ml	
Buffer EB	15 ml	
Power bead	50	
Column Type G (mini) (with collection tube)	50	
1.5 ml microcentrifuge tube	150	

** Before using for the first time, add absolute ethanol (ACS grade or better) into Buffer EW2 as indicated on the bottle.*

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

Product Specifications

Specification	Exgene™ Soil DNA mini
Type	Spin
Maximum amount of starting samples	500 mg soil sample
	47 mm diameter of a nylon membrane filter
	0.5 cm^3 of a air filter
Maximum loading volume of spin column	750 μl
Minimum elution volume	30 μl
Maximum binding capacity	100 μg

QUALITY CONTROL

Exgene™ Soil SV 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

Exgene™ Soil SV should be stored at room temperature (15 °C to 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 56 °C water bath until completely dissolved.

All components are stable for 3 year.

Keep out of direct sunlight.

SAFETY INFORMATION

The buffers included in Exgene™ Soil SV 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

Exgene™ Soil SV is for research use only, not for use in diagnostic procedure.

PREVENTING CONTAMINATION

Proper microbiological, aseptic technique should always be used when working with trace or evidentiary materials. Always wear disposable gloves while handling reagents and samples. The use of sterile tip, tube and other instruments is recommended throughout the procedure.

GeneAll® Exgene™ Soil SV provides a convenient method for the isolation of genomic DNA from soil and environmental 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 organisms in the sample including plant tissues, bacteria, fungi spores and others. Also, it removes a humic acid and other PCR inhibitors from various samples efficiently. The humic acid, which is a sort of brownish color, is a critical factor for soil treating experiments and if remained in eluate, this can have a negative effect on the DNA downstream applications.

Exgene™ Soil SV provides a tube including powerful beads for strong homogenizing. Soil samples are placed in this tube with lysis buffer, Buffer SL and Buffer PD, and crushed by bead-beater or vortex mixer. After centrifugation, supernatant is mixed with precipitation buffer, Buffer RH, 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 EW2, the bound DNA is eluted by Buffer EB. Purified DNA can be directly applicable in conventional PCR, restriction analysis, electrophoresis, or any other downstream applications.

INTENDED USE

Exgene™ Soil SV is designed for the extraction of genomic DNA from various soil and environmental samples, including top soil, mud, sand, granite soil, water filter, and air filter.

PROTOCOL FOR

Exgene™ Soil SV

1. **Add sample to the Power bead as listed below.**

Sample type	Amount
Soil	Up to 500 mg
Water filter (nylon membrane)	One 47 mm diameter filter
Air filter	Up to 0.5 cm^3

2. **Add 550 µl of Buffer SL to Power bead.**

3. **Add 300 µl of Buffer PD to Power bead.**

4. **Homogenize the sample until thoroughly mixed.**
   If using Precellys® 24 (Bertin, France), homogenize twice for 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.

5. **Centrifuge at ≥ 10000 xg for 10 minutes at room temperature and carefully transfer the supernatant to a 1.5 ml microcentrifuge tube (provided).**

6. **Add 50 µl of Buffer RH and mix well by vortexing.**

7. **Centrifuge at ≥ 10000 xg for 5 minutes at room temperature and carefully transfer the supernatant to a 1.5 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 600 µl of Buffer TB and mix well by vortexing.**
   If Buffer TB has precipitated, pre-heat in a 56 °C water bath to dissolve completely.

9. **Transfer up to 750 μl of the mixture to a mini spin column.**

10. **Centrifuge at ≥ 10000 xg for 1 minute at room temperature.**
Discard the pass-through and reinsert the mini spin column back into the same tube.

11. **Repeat step 9~10 with remaining mixture.**

12. **Add 500 μl of Buffer EW2 to the mini spin column.**

13. **Centrifuge at ≥ 10000 xg for 1 minute at room temperature.**
Discard the pass-through and reinsert the mini spin column back into the same tube.

14. **Centrifuge at maximum speed for 2 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 EW2.

15. **Add 50 μl of Buffer EB to the center of the membrane in the mini spin column.**
Incubate for 1 minute at room temperature. Centrifuge at ≥ 10000 xg 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 preferred or the starting materials contain large amount of DNA, elution can be done in 200 μl 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.
Supernatant does not exist after centrifuge homogenized sample in Power bead	**Too much starting material**	Too much starting material can absorb all of lysis buffer. Add more Buffer SL if space is allowed, or reduce the amount of starting material.
Eluate does not perform well in the downstream application	**Residual ethanol remains in eluate**	To remove any residual ethanol included in Buffer EW2 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 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
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	
Forensic	mini	100	122-101	spin / vacuum
		250	122-152	
cfDNA	mini	100	129-101	spin / vacuum

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
Forensic DNA	48	914-048A	tube
	96	914-096A	plate
Cell/Tissue Total RNA	48	915-048A	tube
	96	915-096A	plate
Plant Total RNA	48	916-048A	tube
	96	916-096A	plate
cfDNA	48	917-048A	tube
	96	917-096A	plate

Products	Size	Cat. No.	Type
Fecal DNA/RNA	48	948-048	single
	96	948-096	plate
Forensic	48	936-048	single
	96	936-096	plate
Cell/Tissue Total RNA	48	951-048	single
	96	951-096	plate
Plant Total RNA	48	952-048	single
	96	952-096	plate
cfDNA	48	953-048	single
	96	953-096	plate

GeneAll® AllEx®64 *Compact yet Comprehensive automated extraction system*			
Automatic extraction equipment		AEX064	system
Genomic DNA	48	931-048	single
	96	931-096	plate
Viral DNA/RNA	48	934-048	single
	96	934-096	plate
Blood DNA	48	935-048	single
	96	935-096	plate
Plant DNA/RNA	48	937-048	single
	96	937-096	plate

GeneAll® AllEx®Mini *Compact yet Comprehensive automated extraction system*			
Automatic extraction equipment		AEX012	system
Genomic DNA	48	971-048	single
Viral DNA/RNA	48	972-048	single
Blood DNA	48	973-048	single
Plant DNA/RNA	48	974-048	single
Forensic	48	975-048	single
Fecal DNA/RNA	48	976-048	single
Cell/Tissue Total RNA	48	977-048	single
Plant Total RNA	48	978-048	single

GeneAll

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