Handbook for

GMO SV mini

exgene™

TOTAL DNA PURIFICATION KIT

GeneAll

GeneAll® Exgene™ GMO SV mini

Introduction

This kit can be used to isolate crop DNA that may be used for PCR, southern blotting and etc. Up to 300 mg of crop can be processed with this kit. Phenol/chloroform extraction, alcohol precipitation and cold conditions are not required. This makes it possible to use this kit for simultaneous processing of multiple samples. Eluate in low salt buffer or water is ready for use in downstream applications without further manipulation.

Chemical Hazard

Buffer GP and MB 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.

Quality Control

All components in this kit are manufactured in strict clean condition, and its degree of cleanness is monitored periodically. Restriction enzyme assay, measuring of OD value and conventional or realtime PCR as quality control are carried out from lot to lot thoroughly, and only the qualified is delivered.

Shipping

This kit is shipped at ambient condition.

Storage and Stability

GeneAll® Exgene™ GMO SV mini kit is shipped at room temperature. All solutions should be stored at 15 - 25°C. The kit components are guaranteed to be stable for 18 months from the date of manufacture printed on the product package. Please note that improper storage at 4°C (refrigerator) or -20°C (freezer) will adversely impact DNA purification when precipitates form in the buffers.

The Buffer GL, GP and MB may exhibit salt precipitation at cold temperature due to shipping or lab ambient conditions in winter. The product will not perform optimally if the salt precipitates out of solution. If that happen in any of the solutions, warm the bottles at 50°C above with occasional mixing until completely redissolved.

Kit Contents

Cat. No.	107-150	107-102
Number of Preparation	50	200
GeneAll® Spin Column	50	200
Collection Tubes	100	400
Buffer GL (GMO Lysis Solution)	50 ml	190 ml
Buffer GP (GMO Precipitation Solution)	16 ml	60 ml
Buffer MB (GMO Binding Solution)	35 ml	130 ml
Buffer BW * (Column Wash Buffer B)	30 ml	120 ml
Buffer GW * (Column Wash Buffer G)	60 ml	200 ml
Buffer EB (Elution Buffer)	15 ml	30 ml

** There is no need to add ethanol into Column Wash Buffer.*

GeneAll® Exgene™ GMO SV mini Protocol

Before experiment

- Prepare the water bath to 65°C.

- Buffer GL and MB may precipitate at cool ambient temperature. If so, dissolve it in 50°C water bath.

** Important Note : All centrifugation steps should be performed at ≥10,000 x g (~13,000 rpm) in microcentrifuge at room temperature, unless other directions are mentioned.*

A. *Protocol for grain*

1. **Grind the grains of maize or soybean into a fine powder using a mortar and pestle. Transfer it to a sterile 1.5 ml tube.**
   For example, 300 mg of maize and 120 mg of soybean is optimal amount for one preparation.

2. **Add 800 ul of Buffer GL and vortex for 20 sec to wet the powder thoroughly.**

2a. ***(Optional :)*** **If RNA-free DNA is required, add 20 ul of RNase solution(20 mg/ml, Not provided) and vortex to mix well. Incubate for 3 min at room temperature.**
   GeneAll® Exgene™ GMO SV mini spin column has the much stronger affinity to DNA than RNA. Unless RNase is treated, RNA occupies very small portion of eluates. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR itself.

3. **Incubate at 65°C for 20 min.**

4. **Vortex the mixture for 20 sec, centrifuge for 10 min, and transfer 400 ul of the supernatant to a 1.5 ml tube.**

5. **Add 130 ul of Buffer GP and vortex vigorously for 20 sec.**
   This step is for precipitation of detergent, proteins, and polysaccharides.

6. **Centrifuge for 10 min, and transfer supernatant to a 1.5 ml tube.**
   After centrifugation, the precipitated proteins will form a white pellet.

7. **Add 600 ul of Buffer MB and gently mix by inverting.**

8. **Apply 700 ul of the mixture from step 7 to the spin column sitting in collection tube. Centrifuge for 30 sec, and discard flowthrough.**
   Reuse the colletion tube in step 9.

9. **Repeat step 8 with remaining sample. Discard flowthrough and collection tube.**

10. **Place Spin Column in a new 2 ml collection tube, apply 500 ul Buffer BW to the Spin Column, centrifuge for 1 min and discard flowthrough.**
    Reuse the collection tube in next step.

11. **Add 700 ul of Buffer GW to the Spin Column and centrifuge for 30 sec.**

12. **Remove the spin column, discard the flowthrough, and re-insert the spin column to the collection tube.**

13. **Centrifuge for an additional 2 min to remove residual wash buffer. Transfer the spin column to a new 1.5ml tube (Not provided).**
    Care must be taken at this step for eliminating the carryover of buffer GW. If carryover of buffer GW still occurs, centrifuge again for 1 min before proceeding to next step.

14. **Add 30 ul of Buffer EB directly onto the center of spin column membrane. Incubate for 1 min at room temperature and centrifuge for 1 min.**

B. *Protocol for processed food (plus!* kit only)

1. **Add 300 mg of processed food (wet weight) to 1.5 ml microcentrifuge tube, add 800 ul of Buffer GL and homogenize thoroughly.**
   Various alternative methods can be employed at this step. Small homogenizer can be used with 1.5 ml tube. Much larger amount of sample can be homogenized using equipment as follows and the volume of Buffer GL should be increased proportionally.

   * Glass Dounce homogenizer with Teflon plungers
   * Blenders with rotating blades.
   * Laboratory and Domestic kitchen blenders: when using this minimal amount water should be used at first and then Buffer GL is added.

2. ***(Optional :)* If RNA-free DNA is required, add 20 ul of RNase solution (20 mg/ml, Not provided) and vortex to mix. Incubate for 3 min at room temperature.**
   Please read the annotation of step 2a of 'Protocols for Grain'

3. **Incubate at 65°C for 30 min.**

4. **Vortex the mixture for 20 sec, centrifuge for 10 min, and transfer 500 ul of the supernatant to a 1.5 ml tube.**

5. **Add 160 ul of Buffer GP, vortex vigorously for 20 sec.**
   This step is for precipitation of detergent, proteins, and polysaccharides.

6. **Centrifuge for 10 min. Transfer supernatant to a new 1.5 ml microcentrifuge tube.**

7. ***(Optional :)* Apply the mixture from step 6 to Filter Column (supplied in *plus!* kit) sitting in 2 ml collection tube (supplied in *plus!* kit), centrifuge for 2 min, and discard Filter Column. Transfer the pass-through into a fresh 1.5 ml microcentrifuge tube.**
   This step is necessary for sensitive applications. For routine work such as PCR, there is no need to do this step.

8. **Add 750 ul of Buffer MB to the mixture from previous step and mix by vortexing.**

9. **Apply 700 ul of the mixture from step 8 to the spin column. Centrifuge for 30 sec, and discard flowthrough.**
   Reuse the collection tube in step 9.

10. **Repeat step 9 with remaining sample. Discard flowthrough and collection tube.**

11. **Place spin column in a new 2 ml collection tube (provided), add 500 ul Buffer BW to the spin column, centrifuge for 1 min and discard flowthrough.**

12. **Add 700 ul of Buffer GW to the Spin Column and centrifuge for 30 sec.**

13. **Remove the spin column, discard the flowthrough, and re-insert the spin column to the collection tube.**

14. **Centrifuge for an additional 2 min to remove residual wash buffer. Transfer the spin column to a new 1.5ml tube (Not provided).**
    Care must be taken at this step for eliminating the carryover of buffer GW. If carryover of buffer GW occurs, centrifuge again for an additional 1 min before proceeding to next step.

15. **Add 30 ul of Buffer EB directly onto the center of spin column membrane. Incubate for 1 min at room temperature and centrifuge for 1 min.**

Expected DNA yield and purity

Several micrograms (<10 ug) of DNA can be obtained from a grain of crops, and its value of A_{260}/A_{280} is between 1.7 and 1.9.

Quality Control

DNA was prepared from a grain of soybean or maize. Isolated DNA was tested for quality and function as follows

* Presence of high molecular weight DNA : The DNA was treated with RNase and examined by electrophoresis on an agarose gel. Purified DNA ranges in size up to 30 kb, with fragments of approximately 20 kb predominating.
* Enzymatic reactions are performed routinely including PCR, restriction digestions, etc.
* All solutions and buffers in this kit are manufactured with nuclease free, pyrogen free water (Milli-Q *Biocell*), and are free of contaminating materials.

Typical Experimental Result

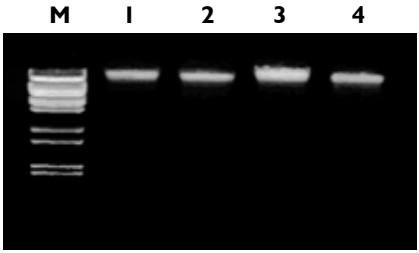

Fig.1 *Soybean genomic DNA. Each lane represents one sixth quantity of isolated genomic DNA from a grain of soybean. M : DNA/Bst P1 Marker, lane 1~4 : soybean genomic DNA purified using this kit*

Ordering Information

Products	Size	Type	Cat. No.
GeneAll® *Hybrid-Q™* *for rapid preparation of plasmid DNA*			
Plasmid Rapidprep	50 200	mini / spin	100-150 100-102
GeneAll® *Exprep™* *for preparation of plasmid DNA*			
Plasmid SV mini	50 200 1,000	spin / vacuum	101-150 101-102 101-111
Plasmid SV Midi**	26 50 100	spin / vacuum	101-226 101-250 101-201
Plasmid SV Quick	50 200 1,000	mini / spin	101-050 101-002 101-011
GeneAll® *Exfection™* *for preparation of highly pure plasmid DNA*			
Plasmid LE mini *(Low Endotoxin)*	50 200	spin / vacuum	111-150 111-102
Plasmid LE Midi* *(Low Endotoxin)*	26 100	spin / vacuum	111-226 111-201
Plasmid EF Midi* *(Endotoxin Free)*	20 100	spin	121-220 121-201
GeneAll® *Expin™* *for purification of fragment DNA*			
Gel SV	50 200	mini / spin / vacuum	102-150 102-102
PCR SV	50 200	mini / spin / vacuum	103-150 103-102
CleanUp SV	50 200	mini / spin / vacuum	113-150 113-102
Combo GP	50 200	mini / spin / vacuum	112-150 112-102
GeneAll® *Exgene™* *for isolation of total DNA*			
Tissue SV mini (*plus!*)*	100 250	spin / vacuum	104(9)-101 104(9)-152
Tissue SV Midi (*plus!*)**	26 100	spin / vacuum	104(9)-226 104(9)-201
Tissue SV MAXI (*plus!*)**	10 26	spin / vacuum	104(9)-310 104(9)-326
Blood SV mini	100 250	spin / vacuum	105-101 105-152
Blood SV Midi**	26 100	spin / vacuum	105-226 105-201

Ordering Information

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

** 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.

Ordering Information

Products	Size	Type	Cat. No.
GeneAll® *RNA Series* *for preparation of total RNA*			
RiboEx™	100 200	solution	301-001 301-002
Hybrid-R™	100	spin	305-101
RiboEx™ LS	100 200	solution	302-001 302-002
Riboclear™	50	spin	303-150
Ribospin™	50	spin	304-150
Ribospin vRD™	50	spin	302-150
Allspin™	50	spin	306-150
GeneAll® *AmpONE™* *for PCR amplification*			
Taq DNA polymerase	250 U 500 U 1,000 U	(2.5 U/μℓ)	501-025 501-050 501-100
α-Taq DNA polymerase	250 U 500 U 1,000 U	(2.5 U/μℓ)	502-025 502-050 502-100
Pfu DNA polymerase	250 U 500 U 1,000 U	(2.5 U/μℓ)	503-025 503-050 503-100
Hot start Taq DNA polymerase	250 U 500 U 1,000 U	(2.5 U/μℓ)	531-025 531-050 531-100
Clean Taq DNA polymerase	250 U 500 U 1,000 U	(2.5 U/μℓ)	551-025 551-050 551-100
Clean α-Taq DNA polymerase	250 U 500 U 1,000 U	(2.5 U/μℓ)	552-025 552-050 552-100
Taq Master mix	2x	0.5 ml x 2 tubes	511-010
α-Taq Master mix	2x	0.5 ml x 2 tubes	512-010
Taq Premix	20 μℓ 50 μℓ	96 tubes	521-200 521-500
α-Taq Premix	20 μℓ 50 μℓ	96 tubes	522-200 522-500
dNTP mix	500 μℓ	2.5 mM each	509-020
dNTP set (set of dATP, dCTP, dGTP and dTTP)	1 ml x 4 tubes	100 mM	509-040

* Each dNTP is available

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