

Handbook for

■ PLANT SV MINI
PLANT SV MIDI
PLANT SV MAXI

exgene[™]

DNA PURIFICATION HANDBOOK

Customer & Technical Support

Should you have any further questions, do not hesitate to contact us.

We appreciate your comments and advice.

Contact Information

www.geneall.com

Tel : 82-2-407-0096

Fax : 82-2-407-0779

E-mail (Order/Sales) : sales@geneall.com

E-mail (Tech. Info.) : tech@geneall.com

Visit GeneAll® Community

www.geneall.com

Trademarks

Hybrid-Q™, Exprep™, Exfection™, Expin™, Exgene™, GenEx™, DirEx™, Hybrid-R™, RiboEx™, Ribospin™, Riboclear™, Allspin™, RiboSaver™, EzClear™, EzSep™, EzPure™, EzPass™, AmpONE™, AmpMaster™, RealAmp™, HyperScript™, ProtinEx™, PAGESTA™, STEADi™, GENTi™, SolMate™ are trademarks of GeneAll Biotechnology Co., Ltd.

© 2022 GeneAll Biotechnology, all rights reserved.

This protocol handbook is included in :

GeneAll® Exgene™ Plant SV mini (117-101, 117-152)

GeneAll® Exgene™ Plant SV Midi (117-226, 117-201)

GeneAll® Exgene™ Plant SV MAXI (117-310, 117-326)

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

INDEX

Kit Contents	04
Storage Conditions	05
Quality Control	
Chemical Hazard	
Product Specifications	06
Exgene™ Plant SV	
Introduction	07
General Considerations	
Starting sample amount	08
Sample preparation, pulverization and lysis	09
Filtration after lysis	10
Elution	11
Centrifuge in Midi/MAXI kits	12
Protocol	
Protocol for Plant SV mini	13
Protocol for Plant SV Midi	16
Protocol for Plant SV MAXI	19
Troubleshooting Guide	22
Ordering Information	24

KIT CONTENTS

Plant SV mini			Plant SV Midi	
Cat. No.	117-101	117-152	117-226	117-201
Size	mini	mini	Midi	Midi
No. of preparation	100	250	26	
SV Column Type G (with collection tube)	100	250	26	117-226 x 4
EzSep™ Filter Column (with collection tube)	100	250	26	
Buffer PL	100 ml	200 ml	100 ml	
Buffer PD	30 ml	90 ml	30 ml	
Buffer BD (concentrate) *	37 ml	51 ml x 2	37 ml	
Buffer CW (concentrate) * †	30 ml	40 ml x 2	50 ml	
Buffer AE **	60 ml	120 ml	60 ml	
RNase A (100 mg/ml)	0.48 ml	1.3 ml	0.48 ml	
Protocol Handbook	1	1	1	

Plant SV MAXI		
Cat. No.	117-310	117-326
Size	MAXI	MAXI
No. of preparation	10	26
SV Column Type G (with collection tube)	10	26
EzSep™ Filter Column (with collection tube)	10	26
Buffer PL	100 ml	200 ml
Buffer PD	30 ml	90 ml
Buffer BD (concentrate) *	37 ml	51 ml x 2
Buffer CW (concentrate) * †	50 ml	50 ml x 3
Buffer AE **	60 ml	120 ml
RNase A (100 mg/ml)	0.48 ml	1.3 ml
Protocol Handbook	1	1

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

† Contains sodium azide as a preservative

** 10 mM TrisCl, pH 9.0, 0.5 mM EDTA

Storage Conditions

All components of GeneAll® Exgene™ Plant SV kit should be stored at room temperature (15~25°C). RNase A is delivered under ambient conditions and can be stored at room temperature for 6 months without significant decrease in activity. But for prolonged conservation of activity, storing at -20~-8°C is recommended.

During delivery or storage under cold ambient condition, a precipitate may be formed in Buffer PL. Heat the bottle to dissolve completely before use. Using precipitated buffers will lead to poor DNA recovery. Exgene™ Plant SV kit series are guaranteed for 1 year.

Quality Control

All components of GeneAll® Exgene™ Plant SV kit are manufactured in strictly clean condition, and its degree of cleanness is monitored periodically. Restriction enzyme assay, PCR amplification assay and spectrophotometric assay as quality control are carried out from lot to lot thoroughly, and only the qualified is delivered.

Chemical Hazard

The buffers included in GeneAll® Exgene™ Plant SV 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.

Buffer BD 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 Specifications

Exgene™ Plant SV	mini	Midi *	MAXI *
Sample amount	~100 mg wet	~400 mg wet	~1 g wet
Preparation time	<40 min	<1 hour	<1 hour
Maximum loading volume	750 μ l	5 ml	15 ml
Binding capacity	50 μ g	170 μ g	400 μ g
Typical yield	4-40 μ g	10-150 μ g	40-300 μ g
Elution volume	30-400 μ l	200-600 μ l	0.4-2 ml

* Exgene™ Plant SV Midi/MAXI kit procedures require the centrifuge which has a swinging-bucket rotor and ability of 4,000~5,000 x g.

■ Introduction

Exgene™ Plant SV kit provides a simple and easy method for the small, medium and large scale purification of total DNA from various plant tissues.

With EzSep™ Filter and SV Column Type G, several plant metabolites are efficiently removed and the procedure can be done in just 40 minutes (mini), yielding a pure DNA suitable for various downstream applications without further manipulation. Up to 100 mg, 400 mg and 1,000 mg of plant tissue can be processed with Exgene™ Plant SV mini, Midi and MAXI, respectively. Exgene™ Plant SV procedure eliminates the need of organic solvent extraction and alcohol precipitation, allowing safe and fast preparation of many samples simultaneously. Purified total DNA can be directly applicable in conventional PCR, real time PCR, Southern blotting, SNP genotyping, RFLP, AFLP and RAPD.

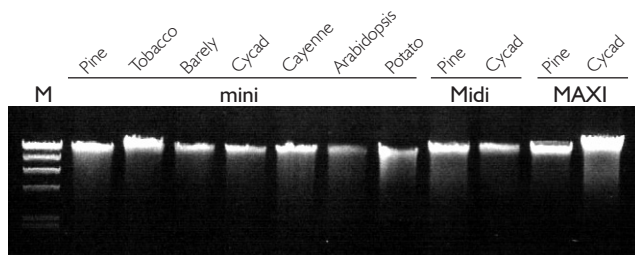


Fig. 1 Genomic DNA prepared from various plant tissues using Exgene™ Plant SV series. Purified DNA was resolved on 0.7% agarose gel.

Scale	mini	Midi	MAXI
Sample weight	100 mg	400 mg	1,000 mg
Elution vol.	100 µl	400 µl	1,000 µl
Loaded vol.	5 µl	5 µl	10 µl

Source	DNA yield (µg)
Arabidopsis	2~5
Barely	4~10
Cayenne	4~18
Cycad	4~15
Maize	7~16
Pine	6~20
Potato	2~8
Soybean	3~15
Tobacco	7~25

Typical yield from various plant tissues (100 mg) with Exgene™ Plant SV mini kit DNA yields vary depending on several factors; age, regions, genome size, stored conditions, and harvest or disruption methods of plant tissue. Midi procedures may yield usually DNA of 3~4 times to mini, and approximately 10 times with MAXI.

General Considerations

■ Starting sample amount

There is an optimized sample amount for Exgene™ Plant SV kit procedures. For mini kit, 100 mg (wet weight) of starting sample material is optimized for the procedures. For dried or lyophilized tissue, it is 25 mg. If the size of starting sample is larger than the optimized, tissue lysis can not be performed efficiently, and this will bring about poor DNA recovery. For large amount of sample, Exgene™ Plant SV Midi/MAXI is available.

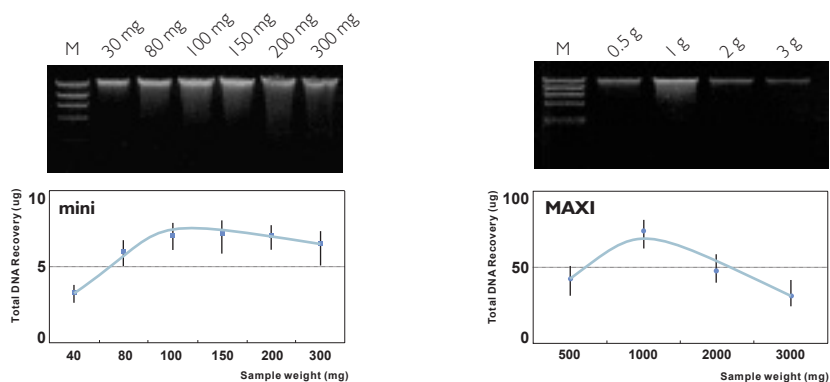


Fig. 2 DNA Preparation from pine.

Use of an excessive starting sample may cause incomplete lysis of sample tissues and the shearing of DNA, resulting in low yield and poor quality of DNA. 2 µl out of 100 µl eluate was resolved on 0.8% agarose gel. M : Lambda-HindIII

■ Sample preparation, pulverization and lysis

When purifying DNA from plants, harvest and pulverization of sample is the most important step for good result. Harvested plant sample or ground tissue powder should be stored under -70°C after frozen in liquid nitrogen for future use. Lyophilized tissue can be stored at room temperature. Fresh and young plant tissues would be best for high yield and good quality of DNA.

Before lysis, tissue sample should be disrupted completely for efficient lysis, and this step should be performed at low temperature (below 0°C) as quickly as possible for optimized result. Lyophilized tissue can be ground at ambient condition.

Mortar and pestle with liquid nitrogen is a typical and good method for grinding of sample. Rotor-stator homogenizer or bead-beater can be a good alternative. Complete and quick pulverization of sample tissue will guarantee the optimized result, while incomplete ground sample or the sample thawed by delayed or poor handling may result in low yields and degraded DNA.

After the addition of Buffer PL, no clumps should be visible in the sample mixture. Because clumped tissue may not lyse appropriately and therefore leads to a low yield of DNA, homogenization by vortexing or pipetting should be carried out for good result. For typical preparations from leaf tissue, lysis at 65°C for 10~15 minutes would be sufficient. Occasional mixing by shaking or inverting of sample tube accelerates the lysis of cells. Incubation in shaking water bath or equivalents would be the best. Lysis time can be prolonged depending on the tissue type used, but it may be sufficient to incubate for 10~20 minutes in most case.

■ Filtration after lysis

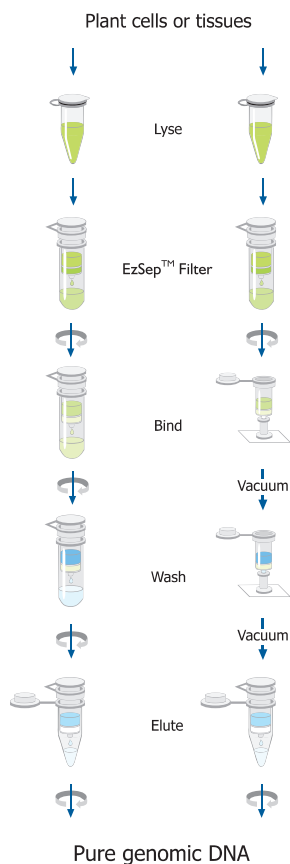
After tissue lysis, the lysate has some debris and salt precipitates, and these should be removed from the lysate to avoid clogging of SV column at binding step. In traditional methods, cell debris and salt precipitates are discarded through pelleting by centrifugation. Traditional methods require rapid and accurate handling of samples to prevent the pellets from loosening, and make it so difficult to prepare many samples simultaneously. Moreover in case of some plant samples, the pellets are not formed tightly, and this may lead the DNA preparation to poor result. EzSep™ Filter included in Exgene™ Plant SV kit makes the preparation of cleared lysate very simple and easy, and facilitates the simultaneous preparation from multiple samples.

In case of some plants, lysate becomes very viscous or sticky after cell lysis, and this leads to shearing of DNA or clogging of EzSep™ Filter. We recommend the optional centrifugation in step 4 in Exgene™ Plant SV mini (page 14) to avoid it.

Plant SV Kit Procedures

in microcentrifuges

on vacuum manifolds



■ Elution

Purified DNA can be eluted in low salt buffer or deionized water depending on the downstream applications. Buffer AE contains 0.5 mM EDTA and 10 mM TrisCl, pH 9.0. The volume of elution buffer can be adjusted, but it has to be over the minimum requirement. To get higher concentration of DNA, decrease the volume of elution buffer to minimum. For higher overall yield, increase the volume of elution buffer and repeat the elution step again. Optimal yields may be obtained by eluting twice. The concentration and yield in relation to the volume of eluent is shown below.

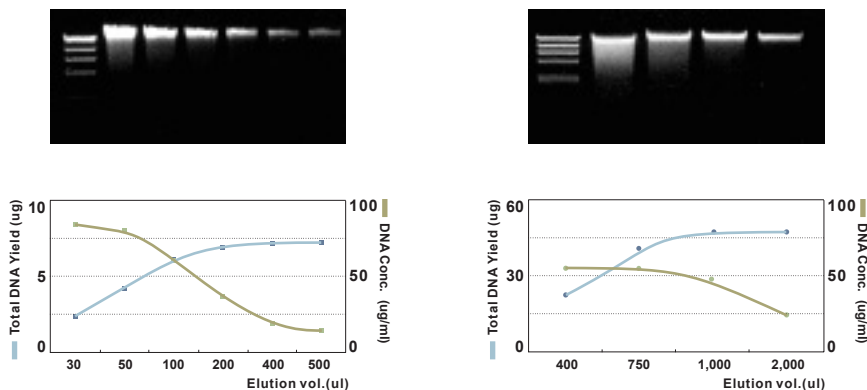


Fig. 3 A series of elution volume was applied to DNA purification from 100 mg of pine leaves for mini procedures and 1 g of cayenne leaves for MAXI procedures. Each 5 μ l of eluate was resolved on 0.8% agarose gel. If the elution volume is reduced for higher concentration of eluate, overall yield will be decreased, especially when the elution volume is below 50 μ l for mini, 200 μ l for Midi, and 500 μ l for MAXI.

■ Centrifuge in Midi/MAXI kits

Exgene™ Plant SV Midi and MAXI procedures require the conventional centrifuge which has a swinging-bucket rotor and ability of 4,000~5,000 x g. Use of fixed-angle rotor will cause inconsistent contact of SV column membrane with mixtures and/or buffers. Low g-force may lead to incomplete removal of ethanol from SV column membrane. Available centrifuges and rotors are listed below, but you can employ any equivalent.

Company	Centrifuge	Rotor
Beckman Coulter Inc. (California, USA)	Allegra X-15R	Sx4750
	Allegra 25R	Sx4750A
		TS-5.1-500
Eppendorf AG (Hamburg, Germany)	5804 / 5804R	A-4-44
	5810 / 5810R	
EYELA Inc. (Tokyo, Japan)	5800	RS-410
	5900	RS-410M
Hanil Science Industrial Inc. (Incheon, Korea)	Union 5KR	R-WS1000-6B
	Union 55R	W-WS750-6B
	MF-550	HSR-4S
	HAI000-6	WHSR-4S
	HAI000-3	
Hettich AG (Kirchlengern, Germany)	Rotina 35	1717
	Rotanta 460	1724
	Rotixa 50S	5624

Before experiment

- Before using for the first time, add absolute ethanol (ACS grade or better) into Buffer BD and CW as indicated on the bottle.
- Unless there is an other indication, all centrifugation steps should be performed at full speed ($> 10,000 \times g$ or 10,000~14,000 rpm) in a microcentrifuge at room temperature.
- Buffer PL may precipitate upon storage at cold ambient temperature. If so, dissolve it in 65°C water bath.

■ Prepare the below;

- » 65°C water bath or heating block
- » 1.5 ml and 2.0 ml microcentrifuge tubes
- » Microcentrifuge

- 1. Grind fresh or frozen plant tissue to a fine powder quickly and completely, using a mortar and pestle pre-cooled with liquid nitrogen. Place up to 100 mg (wet) or 25 mg (dried) of ground tissue into a 1.5 ml or 2.0 ml microcentrifuge tube.**

Quick and complete disruption of tissue is essential for good result in preparation. Grinding under liquid nitrogen is the best method for good result, however other methods such as bead-beater or rotor-stator homogenizer can be a good alternative. Lyophilized tissue can be ground at room temperature.

- 2. Add 400 µl of Buffer PL and 4 µl of RNase A solution (100 mg/ml, provided). Vortex vigorously.**

Any clumps should not be visible. Mix the lysate by pipetting or vortexing to remove any tissue clumps.

- 3. Incubate for 10~15 min at 65°C. Mix 2~3 times during incubation by inverting or vortexing.**

Occasional mixing will accelerate the lysis.

- 4. Add 140 μ l of Buffer PD to the lysate. Vortex to mix, and incubate for 5 min on ice.**

(Optional :) Centrifuge for 5 min at full speed ($> 10,000 \times g$ or 14,000 rpm).

For some plants, the lysate becomes very viscous or sticky after addition of Buffer PD, and this leads to shearing of DNA or clogging of EzSep™ Filter. In this case, removal of precipitates by optional centrifugation will be helpful before proceeding to next step.

- 5. Apply the lysate to the EzSep™ Filter (blue) and centrifuge for 2 min at full speed.**

It may be requisite to use [Wide-bore Tip] or to cut the end off the pipet tip to apply the viscous lysate to the EzSep™ Filter. Small pellet can be formed in the collection tube after centrifugation. Be careful not to disturb this pellet in next step 6.

- 6. Transfer the pass-through to a new 1.5 ml microcentrifuge tube by pipetting or decanting carefully not to disturb the cell debris pellet.**

About 450 μ l of lysate is recovered typically. Recovered volume of lysate can be varied depending on the plant tissue used. Check the correct volume of lysate for optimal binding condition in next step.

- 7. Add 1.5 volumes of Buffer BD to the lysate and mix immediately by pipetting or inverting.**

Adjust the volume of Buffer BD on the basis of correct volume of lysate. For 450 μ l lysate, add 675 μ l Buffer BD. Immediate mixing is important for optimal binding conditions.

A precipitate can be formed after addition of Buffer BD but this will not affect the preparation.

- 8. Apply 700 μ l of the mixture from step 7 to the SV column (green) sitting in collection tube. Centrifuge for 30 sec, and discard the pass-through. Reuse the collection tube.**

Any precipitate which may have formed in mixture should be included in transfer.

- 9. Repeat step 8 with remaining sample.**

- 10. Apply 700 μ l Buffer CW to the SV column, centrifuge for 30 sec and discard the pass-through, and re-insert the SV column to the collection tube.**

- 11. Add 300 μ l of Buffer CW to the SV column. Centrifuge for 2 min. Transfer carefully the SV column to a new 1.5 ml microcentrifuge tube (not provided).**

Care must be taken at the removal of SV column from the collection tube so the column does not come into contact with the pass-through fraction, as this will result in carryover of ethanol.

Residual ethanol in eluate may interfere with the subsequent reactions. If carryover of Buffer CW occurs, centrifuge again for 1 min before proceeding to next step.

- 12. Add 100 μ l of Buffer AE directly onto the center of SV column membrane. Incubate for 5 min at room temperature and centrifuge for 1 min.**

Elution volume can be decreased to 50 μ l 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 μ l of Buffer AE.

- 13. Repeat step 12.**

More 20~40% DNA can be obtained by repeat of eluting.

A new 1.5 ml microcentrifuge tube can be used to prevent dilution of the first eluate.

- Before experiment**
- Before using for the first time, add absolute ethanol (ACS grade or better) into Buffer BD and CW as indicated on the bottle.
 - All centrifugation should be performed at room temperature.
 - Buffer PL may precipitate upon storage at cold ambient temperature. If so, dissolve it in 65 °C water bath.

■ **Prepare the below;**

- » 65 °C water bath or heating block
- » 15 ml conical tubes
- » Centrifuge capable of 4,000~5,000 x g, which has a swinging-bucket rotor (See page 12)
- » The equipment and reagent for tissue disruption; Liquid nitrogen, mortar and pestle

- 1. Grind fresh or frozen plant tissue to a fine powder quickly and completely, using a mortar and pestle pre-cooled with liquid nitrogen. Place up to 400 mg (wet) or 100 mg (dried) of ground tissue into a 15 ml conical tube.**

Quick and complete disruption of tissue is essential for good result in preparation. Grinding under liquid nitrogen is the best method for good result, however other methods such as bead-beater or rotor-stator homogenizer can be good alternatives. Lyophilized tissue can be ground at room temperature.

- 2. Add 2 ml of Buffer PL and 15 µl of RNase A solution (100 mg/ml, provided). Vortex vigorously.**

Any clumps should not be visible. Mix the lysate by pipetting or vortexing to remove any tissue clumps.

- 3. Incubate for 15~20 min at 65 °C. Mix 3~4 times during incubation.**
Occasional mixing will accelerate the lysis.

4. Add 700 μ l of Buffer PD to the lysate. Vortex to mix, and incubate for 10 min on ice.

5. Centrifuge for 5 min at 4,000 x g and carefully decant or pipet the supernatant to the EzSep™ Midi Filter (green ring).

Some debris or salt precipitates can be co-transferred.

6. Centrifuge for 5 min at 4,000 x g. Transfer the filtrate to a new 15 ml conical tube by pipetting or decanting carefully not to disturb the cell debris pellet.

Typically about 2.5 ml of lysate is recovered. Recovered volume of lysate can be varied depending on the plant tissue used. Check the correct volume of lysate for optimal binding condition in next step.

7. Add 1.5 volumes of Buffer BD to the lysate and mix by pipetting or inverting.

Adjust the volume of Buffer BD on the basis of correct volume of recovered lysate. For 2.5 ml lysate add 3.75 ml Buffer BD. Immediate mixing is important for optimal binding conditions.

A precipitate can be formed after addition of Buffer BD but this will not affect the preparation.

8. Apply 4 ml of the mixture including any precipitate which may have formed from step 7 to SV Midi column (white ring). Centrifuge for 2 min at 4,000 x g, discard the filtrate, and reinsert the SV Midi column to the 15 ml conical tube.

Any precipitate which may have formed in mixture should be included in transfer.

9. Repeat step 8 with the remaining sample.

10. Apply 4.5 ml of Buffer CW to the SV Midi column, centrifuge for 2 min at 4,000 x g and discard the filtrate, and re-insert the SV Midi column to the 15 ml conical tube.

- 11. Add 2 ml Buffer CW to the SV Midi column. Centrifuge for 15 min at 4,500 x g. Transfer the SV Midi column to a new 15 ml conical tube (not provided).**

Care must be taken at the removal of SV Midi column from the collection tube so the SV column does not come into contact with the pass-through fraction, as this will result in carryover of ethanol.

Residual ethanol in eluate may interfere with the subsequent reactions. If carryover of ethanol occurs, incubate the Midi column for 15 min at RT to evaporate residual ethanol.

- 12. Add 300 μ l of Buffer AE directly onto the center of SV Midi column membrane. Incubate for 5 min at room temperature and centrifuge for 5 min at 4,000~5,000 x g.**

Elution volume can be decreased to 200 μ l for high concentration of DNA, but this will slightly decrease in overall DNA yield.

- 13. A. For higher concentration of eluate; re-load the eluate from step 12 into the SV Midi column, incubate 5 min at room temperature, and centrifuge for 5 min at 4,000~5,000 x g.**

- B. For higher overall yield; add 300 μ l of fresh Buffer AE into the SV Midi column, incubate 5 min at room temperature, and centrifuge for 5 min at 4,000~5,000 x g.**

The first and second eluate can be combined or collected separately as necessity.

Less than 300 μ l of eluate will be obtained from 300 μ l of elution buffer, but this has no influence on DNA yields.

- Before experiment**
- Before using for the first time, add absolute ethanol (ACS grade or better) into Buffer BD and CW as indicated on the bottle.
 - All centrifugation should be performed at room temperature.
 - Buffer PL may precipitate upon storage at cold ambient temperature. If so, dissolve it in 65°C water bath.

■ **Prepare the below;**

- » 65°C water bath or heating block
- » 50 ml conical tubes
- » Centrifuge capable of 4,000~5,000 x g, which has a swinging-bucket rotor (See page 12)
- » The equipment and reagent for tissue disruption; Liquid nitrogen, mortar and pestle

- 1. Grind fresh or frozen plant tissue to a fine powder quickly and completely, using a mortar and pestle pre-cooled with liquid nitrogen. Place up to 1,000 mg (wet) or 250 mg (dried) of ground tissue into a 50 ml conical tube.**

Quick and complete disruption of tissue is essential for good result in preparation. Grinding under liquid nitrogen is the best method for good result, however other methods such as bead-beater or rotor-stator homogenizer can be good alternatives. Lyophilized tissue can be ground at room temperature.

- 2. Add 5 ml of Buffer PL and 40 µl of RNase A solution (100 mg/ml, provided). Vortex vigorously.**

No clumps should be visible. Mix the lysate by pipetting or vortexing to remove any tissue clumps.

- 3. Incubate for 20 min at 65°C. Mix 3~4 times during incubation time.**

- 4. Add 1.8 ml of Buffer PD to the lysate. Vortex to mix, and incubate for 10 min on ice.**

5. **Centrifuge for 5 min at 4,000 x g and carefully decant or pipet the supernatant to the EzSep™ MAXI Filter (blue).**

Some debris or salt precipitates can be co-transferred.

6. **Centrifuge for 5 min at 4,000 x g and transfer the pass-through to a new 50 ml conical tube by pipetting or decanting carefully not to disturb the cell debris pellet.**

Typically, 5~6 ml of lysate is recovered. Recovered volume of lysate can be varied depending on the plant tissue used. Check the correct volume of lysate for optimal binding condition in next step.

7. **Add 1.5 volumes of Buffer BD to the lysate and mix by pipetting or inverting.**

Adjust the volume of Buffer BD on the basis of correct volume of recovered lysate. For 5 ml lysate add 7.5 ml Buffer BD. Immediate mixing is important for optimal binding conditions.

A precipitate can be formed after addition of Buffer BD but this will not affect the preparation.

8. **Apply the sample mixture including any precipitate which may have formed from step 7 to the SV MAXI column (white). Centrifuge for 2 min at 4,000 x g and discard the pass-through and re-insert the MAXI column to the collection tube.**
9. **Apply 13 ml of Buffer CW to the SV MAXI column, centrifuge for 2 min at 4,000 x g and discard the pass-through, and re-insert the SV MAXI column to the collection tube.**

- 10. Add 5 ml Buffer CW to the SV MAXI column. Centrifuge for 15 min at 4,500 x g. Transfer the SV MAXI column to a new 50 ml conical tube (not provided).**

Care must be taken at the removal of SV MAXI column from the collection tube so the MAXI column does not come into contact with the pass-through fraction, as this will result in carryover of ethanol. Residual ethanol in eluate may interfere with the subsequent reactions. If carryover of ethanol occurs, incubate the MAXI column for 15 min at RT to evaporate residual ethanol.

- 11. Add 0.6~1 ml of Buffer AE directly onto the center of SV MAXI column membrane. Incubate for 5 min at room temperature and centrifuge for 5 min at 4,000~5,000 x g.**

Elution volume can be decreased to 500 μ l for high concentration of DNA, but this will slightly decrease in overall DNA yield.

- 12. A. For higher concentration of eluate; re-load the eluate from step 11 into the SV MAXI column, incubate 5 min at room temperature, and centrifuge for 5 min at 4,000~5,000 x g.**

- B. For higher overall yield; add 0.6~1 ml of fresh Buffer AE into the SV MAXI column, incubate 5 min at room temperature, and centrifuge for 5 min at 4,000~5,000 x g.**

The first and second eluates can be combined or collected separately as necessity.

Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low or no recovery	Too much starting material	Too much starting material lead to inefficient lysis and column clogging, followed by poor DNA yields. Reduce the amount of starting material.
	Too old or mis-stored sample used	Refer “Sample preparation, pulverization and lysis” on page 9.
	Insufficient pulverization	Refer “Sample preparation, pulverization and lysis” on page 9.
	Incorrect binding	Ensure the binding conditions are adjusted correctly in step 7.
	Improper elution	The condition for optimal elution is of low salt concentration with weakly alkaline pH ($7.0 < \text{pH} < 9.0$). Ensure the condition when water or other buffer was used as eluent. After eluent is applied on the center of column membrane, it is essential to incubate at least for 5 min at room temperature.
	Improper centrifuge (Midi/MAXI)	Swinging-bucket rotor (capable of 4,000~5,000 x g) should be used fixed-angle rotor is not compatible with this kit (See page 12).
Low purity	Incomplete precipitation	Any cell debris or precipitates should be removed before addition of Buffer BD.
	Insufficient lysis	Too much starting material can lead to poor lysis, followed by low purity of DNA.
	Improper centrifuge (Midi/MAXI)	Swinging-bucket rotor (capable of 4,000~5,000 x g) should be used fixed-angle rotor is not compatible with this kit (See page 12).

Facts	Possible Causes	Suggestions
Clogging of EzSep™ Filter	High viscosity of lysate (mini)	Perform the optional centrifugation step in step 4 before applying to EzSep™ Filter.
	Insufficient centrifugation (Midi/MAXI)	Increase the g-force and centrifugation time (See page 12).
Clogging of Exgene™ Plant SV column	Incomplete removal of precipitate	Any cell debris or precipitates should be removed before addition of Buffer BD.
	Lysate too viscous or sticky	Reduce the amount of starting sample, or increase the amount of Buffer PL and PD.
	Insufficient centrifugation (Midi/MAXI)	Increase the g-force and centrifugation time (See page 12).
DNA sheared	Too much starting materials	Too much starting material can make the lysate very viscous and lead to shearing of DNA. Reduce the amount of starting material.
	Too old or mis-stored sample used	Refer "Sample preparation, pulverization and lysis" on page 9.
	Too viscous lysate (mini)	In some plants, the lysate may become too viscous, so the optional centrifugation in step 4 should be performed before applying to EzSep™ Filter.
Enzymatic reaction is not performed well with purified DNA	High salt concentration in eluate	Ensure that washing step was carried out just in accordance with the protocols. Repeat of washing step may help to remove high salt in eluate.
	Low purity of DNA	See "Low purity" at page 22.
	Residual ethanol in eluate	Ensure that the wash step in protocols is performed properly. Exgene™ Plant SV column membrane should be completely dried by additional centrifugation or air-drying before elution.

Ordering Information

Products	Scale	Size	Cat. No.	Type
----------	-------	------	----------	------

GeneAll® Hybrid-Q™ for rapid preparation of plasmid DNA

Plasmid Rapidprep	mini	50	100-150	spin
		200	100-102	

GeneAll® Expres™ for preparation of plasmid DNA

Plasmid SV	mini	50	101-150	spin /
		200	101-102	vacuum
	Midi	26	101-226	spin /
		50	101-250	
		100	101-201	

GeneAll® Exfection™ for preparation of transfection-grade plasmid DNA

Plasmid LE (Low Endotoxin)	mini	50	111-150	spin /
		200	111-102	vacuum
	Midi	26	111-226	spin /
		100	111-201	vacuum
Plasmid EF (Endotoxin Free)	Midi	20	121-220	spin
		100	121-201	

GeneAll® Expin™ for purification of fragment DNA

Gel SV	mini	50	102-150	spin /
		200	102-102	vacuum
PCR SV	mini	50	103-150	spin /
		200	103-102	vacuum
CleanUp SV	mini	50	113-150	spin /
		200	113-102	vacuum
Combo GP	mini	50	112-150	spin /
		200	112-102	vacuum

GeneAll® Exgene™ for isolation of total DNA

Tissue SV	mini	100	104-101	spin /
		250	104-152	vacuum
	Midi	26	104-226	spin /
		100	104-201	vacuum
	MAXI	10	104-310	spin /
		26	104-326	vacuum
Tissue plus! SV	mini	100	109-101	spin /
		250	109-152	vacuum
	Midi	26	109-226	spin /
		100	109-201	vacuum
	MAXI	10	109-310	spin /
		26	109-326	vacuum

Products	Scale	Size	Cat. No.	Type
----------	-------	------	----------	------

GeneAll® Exgene™ for isolation of total DNA

Blood SV	mini	100	105-101	spin /
		250	105-152	vacuum
	Midi	26	105-226	spin /
		100	105-201	vacuum
	MAXI	10	105-310	spin /
		26	105-326	vacuum
Cell SV	mini	100	106-101	spin /
		250	106-152	vacuum
	MAXI	10	106-310	spin /
		26	106-326	vacuum
	mini	100	108-101	spin /
		250	108-152	vacuum
Clinic SV	Midi	26	108-226	spin /
		100	108-201	vacuum
	MAXI	10	108-310	spin /
		26	108-326	vacuum
Genomic DNA micro		50	118-050	spin
Plant SV	mini	100	117-101	spin /
		250	117-152	vacuum
	Midi	26	117-226	spin /
		100	117-201	vacuum
	MAXI	10	117-310	spin /
		26	117-326	vacuum
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	

GeneAll® GenEx™ for isolation of total DNA without spin column

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 <i>plus!</i>	Sx	100	228-101	solution
	Mx	50	228-250	
	Lx	20	228-320	

GeneAll® DirEx™ series for preparation of PCR-template without extraction

DirEx™		100	250-101	solution
DirEx™ <i>Fast-Tissue</i>	96 T		260-011	solution
DirEx™ <i>Fast-Cultured cell</i>	96 T		260-021	solution
DirEx™ <i>Fast-Whole blood</i>	96 T		260-031	solution
DirEx™ <i>Fast-Blood stain</i>	96 T		260-041	solution
DirEx™ <i>Fast-Hair</i>	96 T		260-051	solution
DirEx™ <i>Fast-Buccal swab</i>	96 T		260-061	solution
DirEx™ <i>Fast-Cigarette</i>	96 T		260-071	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™ <i>plus!</i>	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 <i>plus!</i>	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/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	

Products	Scale	Size	Cat. No.	Type
----------	-------	------	----------	------

GeneAll® Protein series

ProtinEx™ Animal cell / tissue	100 ml	701-001	solution
PAGESTA™ Reducing 5X SDS-PAGE Sample Buffer	1 ml × 10 tubes	751-001	solution

GeneAll® STEADi™ for automatic nucleic acid purification

12 Instrument		GST012	system
24 Instrument		GST024	system
Genomic DNA Cell / Tissue	96	401-104	kit
Genomic DNA Blood	96	402-105	kit
Total RNA	96	404-304	kit
Viral DNA / RNA	96	405-322	kit
Genomic DNA Plant	96	407-117	kit
Soil DNA	96	408-114	kit

GeneAll® GENTi™ 32 *Ultimately flexible automatic extraction system*

Automatic extraction equipment		GTI032	system
Genomic DNA	48	901-048	tube
	96	901-096	plate
Viral DNA / RNA	48	902-048	tube
	96	902-096	plate
Whole Blood Genomic DNA	48	903-048	tube
	96	903-096	plate

Products	Scale	Size	Cat. No.	Type
----------	-------	------	----------	------

GeneAll® GENTi™ 32 *Ultimately flexible automatic 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
Plant DNA / RNA	48	904-048A	tube
	96	904-096A	plate

Note



GeneAll

GENEALL BIOTECHNOLOGY CO., LTD



GENEALL BIOTECHNOLOGY CO., LTD

GeneAll Bldg., 303-7, Dongnamro, Songpa-gu, Seoul, Korea 05729

E-mail : sales@geneall.com

Tel. 82-2-407-0096 Fax. 82-2-407-0779

www.geneall.com

Manufacturer site

A-1201~A-1204, Hanam Techno Valley UI Center,
947, Hanam-daero, Hanam-si, Gyeonggi-do, 12982, Korea