Ver 2.2 HBI170

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.

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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.

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KIT CONTENTS

	Plant :	SV mini	Plant S	V Midi
Cat. No.	11 <i>7</i> -101	11 <i>7</i> -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	
EzSep™ Filter Column (with collection tube)	100	250	26	
Buffer PL	100 ml	200 ml	100 ml	117-226
Buffer PD	30 ml	90 ml	30 ml	x 4
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 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. ExgeneTM Plant SV kit series are guaranteed for I year.

Quality Control

All components of ExgeneTM 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 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 μl 5 ml		15 ml
Binding capacity	50 µg	170 μg	400 µg
Typical yield	4-40 µg	10- 150 µg	40-300 µg
Elution volume	30-400 µl	200-600 µl	0.4-2 ml

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

Exgene[™] Plant SV

Introduction

Exgene TM 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 EzSepTM 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 ExgeneTM Plant SV mini, Midi and MAXI, respectively. ExgeneTM 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.

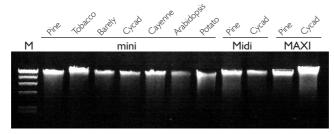


Fig. 1 Genomic DNA prepared from various plant tissues using Exgene[™] Plant SV series. Purified DNA was resolved on 0.7% agarose gel.

Scale	mini	Midi	MAXI
Sample weight	100 mg	400 mg	1,000 mg
Elution vol.	100μ I	400μ l	μ 000, I
Loaded vol.	5 µI	5 µI	Ι0 <i>μ</i> Ι

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 ExgeneTM 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 ExgeneTM 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, ExgeneTM Plant SV Midi/MAXI is available.

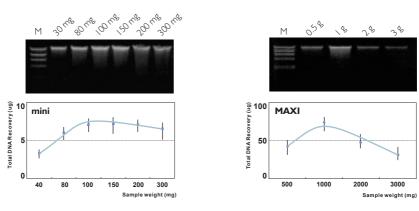


Fig. 2 DNA Preparation from pine.

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 μ l out of 100 μ l 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.

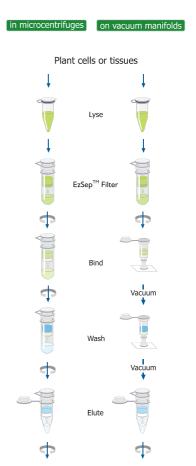
After the addition of Buffer PL, no clumps should be visible in the sample mixture. Because clumped tissue may not lyse appropriately and therefore leads to a low yield of DNA, homogenization by vortexing or pipetting should be carried out for good result. For typical preparations from leaf tissue, lysis at 65° C for $10\sim15$ 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 $10\sim20$ 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. EzSepTM 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 EzSepTM Filter. We recommend the optional centrifugation in step 4 in ExgeneTM Plant SV mini (page 14) to avoid it.

Plant SV Kit Procedures



Pure genomic DNA

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.

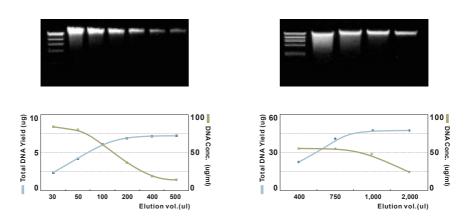


Fig. 3 A series of elution volume was applied to DNA purification from 100 mg of pine leaves for mini procedures and 1 g of cayenne leaves for MAXI procedures. Each 5 µl of eluate was resolved on 0.8% agarose gel. If the elution volume is reduced for higher concentration of eluate, overall yield will be decreased, especially when the elution volume is below 50 µl for mini, 200 µl for Midi, and 500 µl 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 $4,000 \sim 5,000 \times g$. 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 Allegra 25R	Sx4750 Sx4750A TS-5.1-500
Eppendorf AG (Hamburg, Germany)	5804 / 5804R 5810 / 5810R	A-4-44
EYELA Inc. (Tokyo, Japan)	5800 5900	RS-410 RS-410M
Hanil Science Industrial Inc. (Incheon, Korea)	Union 5KR Union 55R MF-550 HA1000-6 HA1000-3	R-WS1000-6B W-WS750-6B HSR-4S WHSR-4S
Hettich AG (Kirchlengern, Germany)	Rotina 35 Rotanta 460 Rotixa 50S	1717 1724 5624

Exgene[™] Plant SV mini

- **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 x g or $10,000 \sim 14,000$ 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
- 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 μ l of Buffer PL and 4 μ l 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.

Incubate for 10~15 min at 65°C. Mix 2~3 times during incu-bation 3. 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 x 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 EzSepTM 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 $EzSep^{TM}$ 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 μl 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 I 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.
- II. Add 300 μ I 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 I 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 $20{\sim}40\%$ 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.

Exgene[™] Plant SV Midi

- **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 $4,000 \sim 5,000 \times g$, which has a swingingbucket rotor (See page 12)
- » The equipment and reagent for tissue disruption; Liquid nitrogen, mortar and pestle
- I. 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 μ l 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.

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

- 4. Add 700 μ I 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 $EzSep^{TM}$ 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 I 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.

Exgene[™] Plant SV MAXI

- **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 $4,000 \sim 5,000 \times g$, which has a swingingbucket rotor (See page 12)
- » The equipment and reagent for tissue disruption; Liquid nitrogen, mortar and pestle
- Grind fresh or frozen plant tissue to a fine powder quickly and ١. 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 μ l 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.

- Incubate for 20 min at 65°C. Mix 3~4 times during incubation 3. time.
- 4. Add I.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, $5\sim6$ 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.
- 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.

II. Add 0.6~I 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~I 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 <ph<9.0). 5="" after="" applied="" as="" at="" buffer="" center="" column="" condition="" eluent="" eluent.="" ensure="" essential="" for="" incubate="" is="" it="" least="" membrane,="" min="" of="" on="" or="" other="" room="" temperature.<="" th="" the="" to="" used="" was="" water="" when=""></ph<9.0).>
	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).
	Incomplete precipitation	Any cell debris or precipitates should be removed before addition of Buffer BD.
Low purity	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™	Incomplete removal of precipitate	Any cell debris or precipitates should be removed before addition of Buffer BD.
Plant SV column	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 TM Filter.
Enzymatic reaction is not performed well with	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.
purified DNA	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.	Туре	Products	Scale	Size	Cat. No.	Туре
GeneAll® Hybrid	I-Q TM fo	r rapid p	reparation of	plasmid DNA	GeneAll® Exgene	TM for is	olation o	f total DNA	
-		50	100-150				100	105-101	spin /
Plasmid Rapidprep	mini	200	100-102	spin		mini	250	105-152	vacuum
		200	100 102		-		26	105-226	spin /
GeneAll® <i>Expre</i>	TM for bi	reparatio	on of plasmid i	DNA	Blood SV	Midi	100	105-201	vacuum
		50	101-150	spin /	-		10	105-310	spin /
	mini	200	101-102	vacuum		MAXI	26	105-326	vacuum
Plasmid SV		26	101-226				100	106-101	spin /
	Midi 50 101-250 spin /	mini	250	106-152	vacuum				
		100	101-201	vacuum	Cell SV		10	106-310	spin /
Com o A II ® Ff+	TM					MAXI	26	106-326	vacuum
GeneAll [®] Exfect	ion iration of	transfect	tion-grade pla	smid DNA			100	108-101	spin /
. , ,		50	111-150	spin /		mini	250	108-152	vacuum
Plasmid LE	mini	200	111-102	vacuum	-		26	108-226	spin /
(Low Endotoxin)		26	111-226	spin /	Clinic SV	Midi	100	108-201	vacuum
,	Midi	100	111-201	vacuum	-		10	108-310	spin /
DI LIEE	ee) Midi	20	121-220			MAXI	26	108-326	vacuum
Plasmid EF (Endotoxin Free)		100	121-201	spin	Genomic DNA micro		50	118-050	spin
GeneAll [®] Expin TM for pu				100	117-101	spin /			
		ification i	of fragment [NA		mini	250	117-152	vacuum
Bellerit Expili	Joi pui	50	102-150				26	117-226	spin /
Gel SV	mini	200	102-130	spin / vacuum	Plant SV	Midi	100	117-201	vacuum
		50	102-102		-	MAXI	10	117-310	spin /
PCR SV	mini	200	103-130	spin / vacuum			26	117-326	vacuum
		50	113-150		Soil DNA mini	mini	50	114-150	spin
CleanUp SV	mini	200	113-130	spin / vacuum	Stool DNA mini	mini	50	115-150	spin
		50	113-102		Stool-Bead DNA mini	mini	50	115-151	spin
Combo GP	mini	200	112-130	spin / vacuum	Viral DNA/RNA	mini	50	128-150	spin
		200	112-102	vacuum			50	138-150	
GeneAll® <i>Exgen</i> e	TM for is	olation o	of total DNA		FFPE Tissue DNA	mini	250	138-152	- spin
Deller III Exgen	101 13			. ,			100	122-101	
	mini	250	104-101	spin / vacuum	Forensic	mini	250	122-152	- spin / vacuur
					dDNA	mini	100	129-101	spin / vacuur
Tissue SV	Midi	26	104-226	spin /					Spirity vacaditi
			104-201	vacuum		for	isolation	of total DNA	4
	MAXI	10	104-310	spin /	GeneAll® GenE x [†]	1//1		column	•
		26	104-326	vacuum			100	220-101	1.2
	mini	250	109-101	spin /	$GenEx^{TM}$ Blood	Sx	500	220-105	- solution
			109-152	vacuum	-	Lx	100	220-301	solution
Tissue Plus SV	Midi	26	109-226	spin /			100	221-101	1.7
		100	109-201	vacuum	$GenEx^{TM}$ Cell	Sx	500	221-105	- solution
	MAXI	10	109-310	spin /	Lx	100	221-301	solution	
			1119_376	vacuum					
		20	107-320				100	222-101	1.0
		20	107-320		GenEx [™] Tissue	Sx	100 500	222-101 222-105	- solution

Products	Scale	Size	Cat. No.	Туре
GeneAll® GenEx	TAA '	solation out spin	of total DNA column	
GenEx [™] Plant	Sx	100	227-101	
	Mx	100	227-201	solution
	Lx	100	227-301	
	Sx	100	228-101	
GenEx [™] Plant Plus	Mx	50	228-250	solution
-	Lx	20	228-320	

GeneAll® DirExTM series
for preparation of PCR-template without extraction

	for preparation of	y i cir con	ipiace manou	c chadeaon
	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	260-03 I	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 preparation of total RNA

$RiboEx^{TM}$	mini	100	301-001	solution
NIDOEX	THILI	200	301-002	SOIULION
Hybrid-R TM	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
NIDOEX L3	THITH	200	302-002	SOIULION
Riboclear™	mini	50	303-150	spin
Riboclear [™] Plus	mini	50	313-150	spin
Ribospin [™]	mini	50	304-150	spin
Dila i - TM II	mini	50	314-150	:-
Ribospin [™] II	THITH	300	314-103	spin
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 [™]		50	314-150	onin
Pathogen/TNA	mini	250	314-152	spin
Allspin [™]	mini	50	306-150	spin
RiboSaver™	mini	100	351-001	solution

Products	Scale	Size	Cat. No.	Туре
GeneAll® A mp	ONE TM fo	r PCR aı	mplification	
		250 U	501-025	
Taq DNA polyme	rase	500 U	501-050	(2.5 U/µI)
		I,000 U	501-100	
To a Donation	20 µl x 9	6 tubes	526-200	solution
Taq Premix	50 μl x 9	6 tubes	526-500	SOIUTION

GeneAll® AmpMasterTM for PCR amplification

Tag Master mix	0.5 ml x 2 tubes	541-010	solution
iaq i iaster mix	0.5 ml x 10 tubes	541-050	solution

GeneAll® HyperScriptTM for Reverse Transcription

Reverse Transcripta	se 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	20μ l × 96 tubes	602-102	solution

GeneAll® RealAmp[™] for qPCR amplification

		,	, ,	
SYBR qPCR Master	200 rxn	2 ml	801-020	solution
$\operatorname{mix}\left(2X,\operatorname{Low}\operatorname{ROX}\right)$	500 rxn	5 ml	801-050	SOIULION
SYBR qPCR Master	200 rxn	2 ml	801-021	and the same
mix(2X,HighROX)	500 rxn	5 ml	801-051	solution

GeneAll® Protein series

ProtinEx [™] Animal cell/tissue	100 ml	701-001	solution
PAGESTA [™] Reducing 5X SDS-PAGE Sample Buffer	I ml x 10 tubes	751-001	solution

Products	Size	Cat. No.	Tupo
Froducts	Size	Cat. No.	Туре
GeneAll® GENTi	и зг Nev aut	wly designed omated extracti	on system
Automatic extraction equipm	nent	GTI032A	system
Genomic DNA	48	901-048A	tube
Genomic DIVA	96	901-096A	plate
Viscal DNIA /DNIA	48	902-048A	tube
Viral DNA/RNA	96	902-096A	plate
	48	903-048A	tube
Blood DNA	96	903-096A	plate
Disast DNIA (DNIA	48	904-048A	tube
Plant DNA/RNA	96	904-096A	plate
LMO	48	906-048A	tube
LMO	96	906-096A	plate
Fecal DNA/RNA	48	913-048A	tube
i ecai DINAYNINA	96	913-096A	plate

	GeneAll®	AllEx [®] 64	Compact yet Comprehensive automated extraction system
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Automatic extraction equipm	nent	AEX064	system
Genomic DNA	48	931-048	tube
Genomic DINA	96	931-096	plate
V/_ DN A /DN A	48	934-048	tube
Viral DNA/RNA	96	934-096	plate
DI IDNIA	48	935-048	tube
Blood DNA	96	935-096	plate
Plant DNA/RNA	48	937-048	tube
	96	937-096	plate
Focal DNIA /DNIA	48	948-048	tube
Fecal DNA/RNA	96	948-096	plate
Forensic	48	936-048	tube
TOTETISIC	96	936-096	plate
Rice DNA	48	949-048	tube
NICE DIVA	96	949-096	plate
Mart Carania DNIA	48	950-048	tube
Meat Genomic DNA	96	950-096	plate

Products	Size	Cat. No.	Туре
Cell/Tissue Total RNA	48	951-048	tube
Cell/ Hissue Total N VA	96	951-096	plate
dDNA	48	953-048	tube
CIDINA	96	953-096	plate
GeneAll® AllEx ® M		npact yet Comp omated extracti	
		orrideed extracti	OII SYSTEIII
Automatic extraction equipm		AEX012	system
Automatic extraction equipmed Genomic DNA	nent 48		
		AEX012	system
Genomic DNA	48	AEX012 971-048	system tube
Genomic DNA Viral DNA/RNA	48 48	AEX012 971-048 972-048	system tube tube
Genomic DNA Viral DNA/RNA Blood DNA	48 48 48	AEX012 971-048 972-048 973-048	system tube tube tube
Genomic DNA Viral DNA/RNA Blood DNA Plant DNA/RNA	48 48 48 48	AEX012 971-048 972-048 973-048 974-048	system tube tube tube tube
Genomic DNA Viral DNA/RNA Blood DNA Plant DNA/RNA Forensic DNA	48 48 48 48 48	AEX012 971-048 972-048 973-048 974-048 975-048	system tube tube tube tube tube

Note





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