

2009
■ **Hybrid-Q™**
Plasmid Rapidprep

DNA PURIFICATION HANDBOOK



hybrid-Q™

Customer & Technical Support

Do not hesitate to ask us any question.

We thank you for any comment or advice.

Contact us at

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

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www.geneall.com

www.geneall.co.kr

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This protocol handbook is included in :

GeneAll® Hybrid-Q™ Plasmid Rapidprep (100-150, 100-102)

Visit www.geneall.com or www.geneall.co.kr for FAQ, QnA and more information.

INDEX

Kit Contents	04
Precautions and Disclaimer	05
Chemical Hazard	
Quality Control	
Storage condition	
Product specifications	06
GeneAll® Hybrid-Q™ Plasmid RapidPrep Kit	
Introduction	07
Principle of Method	
GeneAll® Hybrid-Q™ Quick View	08
General considerations	09
Starting materials	
Alkaline lysis	11
Filtration of lysate with EzClear™ Filter Column	12
Washing	12
Elution	13
Protocols for Hybrid-Q™	
Hybrid-Q™ Rapid protocol	14
Hybrid-Q™ Standard protocol	19
Hybrid-Q™ Low-copy plasmid protocol	24
Troubleshooting Guide	29
Ordering Information	32

KIT CONTENTS

Cat. No.	Hybrid-Q™	
	100-150	100-102
No. of preparations	50	200
Spin column type Q	50	200
EzClear™ filter column	50	200
Buffer S1	20 ml	60 ml
Buffer S2	20 ml	60 ml
Buffer G3	25 ml	90 ml
Buffer AW	30 ml	120 ml
Buffer PW	60 ml	250 ml
Buffer EB*	15 ml	30 ml
RNase A (20 mg/ml)	2 mg	6 mg
Protocol Handbook	1	1

* 10 mM TrisCl, pH 8.5

Precautions and Disclaimer

GeneAll® Hybrid-Q™ Plasmid Rapidprep Kit is for research use only, and should not be used for drug, household or other unintended uses. All due care and attention should be taken in every procedure in this handbook. Please consult Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

Chemical Hazard

The buffers included in GeneAll® Hybrid-Q™ Plasmid Rapidprep Kit contain the irritant which is harmful when in contact with skin, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector, and follow standard safety precautions.

Buffer G3 and AW contain chaotropic salts. It can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solution directly to the sample-preparation waste.

Quality Control

All components in GeneAll® Hybrid-Q™ Plasmid Rapidprep Kit are manufactured in strictly clean condition, and its degree of cleanness is monitored periodically.

Restriction enzyme assay, gene cloning, PCR amplification assay and automated sequencing analysis as quality control are carried out from lot to lot thoroughly, and only the qualified is delivered.

Storage conditions

GeneAll® Hybrid-Q™ Plasmid Rapidprep Kit is shipped at room temperature. All components are stable at room temperature until the date of expiration that is printed on the product label. After addition of RNase A, buffer S1 is stable for 1 year when stored at 4 °C.

In cold ambient condition, buffer S2 and G3 may exhibit salt precipitation and this will cause reduction of DNA recover-yields. If so, heat the bottle with occasional swirling in 37 °C water bath until completely dissolved.

Product Specifications

GeneAll® Hybrid-Q™	
Size	mini
Format	Spin
Recommended sample volume (High copy)	2 ~ 5 ml
Maximum sample volume (Low copy)	10 ml
Maximum loading volume of EzClear™ filter	600 ul
Maximum loading volume of spin column	800 ul
Binding capacity	30 ug
Recover rate	85 ~ 95 %
Minimum elution volume	40 ul

GeneAll[®] Hybrid-Q[™] Plasmid Rapidprep Kit

Introduction

GeneAll[®] Hybrid-Q[™] Plasmid Rapidprep Kit provides two methods for easy and rapid preparation of plasmid DNA from the mini scale bacterial cells. Plasmid DNA can be prepared from up to 10 ml of overnight culture by conventional miniprep method with standard protocol. Alternatively, up to 3 ml of sample can be processed by rapid protocol in just 10 minutes with new patented EzClear[™] filter and simultaneous processing of multiple samples can be easily performed.

This kit can be used to isolate and purify any plasmid, but works most efficiently when the plasmid is less than 20 kb in size.

Up to 30 ug of pure plasmid can be purified using GeneAll[®] Hybrid-Q[™] Plasmid Rapidprep Kit and this pure plasmid DNA is ready for PCR, cloning, fluorescent sequencing, synthesis of labeled hybridization probes, cell transfection, electroporation, and enzymatic restriction analysis without further manipulation.

Principle of Method

GeneAll[®] Hybrid-Q[™] Plasmid Rapidprep Kit utilizes glass microfiber membrane based on the modified alkaline lysis method. Alkaline lysis releases plasmid DNA from bacterial cells and degrades RNA, and RNase removes any survived RNA in the lysate. Cell debris and salt precipitates are removed by EzClear[™] filter or conventional centrifugation.

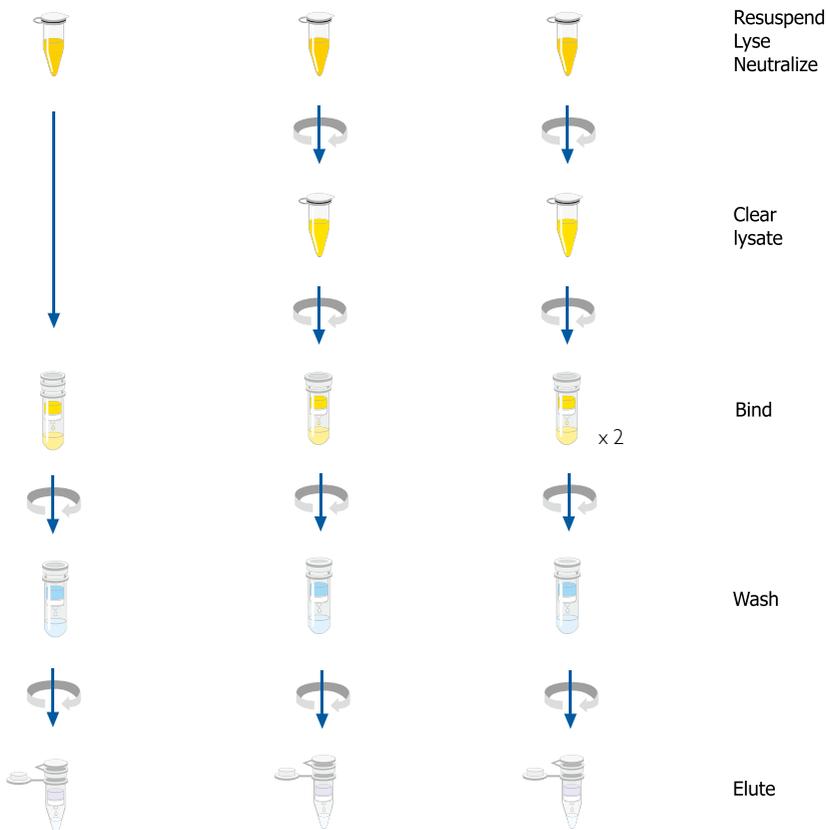
In the presence of high salt, plasmid DNA in the cleared lysate binds selectively to glass microfiber membrane in GeneAll[®] spin column. Bound plasmid DNA is purified in a series of washing steps to eliminate contamination of other bacterial components. Finally elution by low salt buffer or deionized water releases plasmid DNA from the glass microfiber membrane. This simple method eliminates the need for organic solvent extraction and alcohol precipitation.

GeneAll[®] Hybrid-Q[™] Quick View

Hybrid-Q[™] Rapid

Hybrid-Q[™] Standard

Hybrid-Q[™] Low-copy



High-purity plasmid DNA

General considerations

■ Starting material

The yield and quality of plasmid DNA depends on several factors such as plasmid copy number, bacterial strain, antibiotics, inoculation and type of culture medium.

Whenever possible, plasmids should be purified from bacterial cultures that have been inoculated with a single transformed colony picked from an agar plate.

Usually, the colony is transferred to a small starter culture, which is grown to late log phase. Aliquots of this culture can be used to prepare small amounts of the plasmid DNA for analysis and/or as the inoculum for a large-scale culture. The conditions of growth of the large-scale culture depend chiefly on the copy number of the plasmid and whether it replicates in a stringent or relaxed fashion. At all times, the transformed bacteria should be grown in selective conditions, i.e., in the presence of the appropriate antibiotics.

The copy number of a plasmid is defined as the average number of plasmids per bacterial cells under normal growth conditions. Plasmids have own copy number per cell, depending on their origin of replication (replicon) and the size of plasmid DNA. A plasmid replicon can be defined as the smallest piece of plasmid DNA that is able to replicate autonomously and maintain normal copy number by determining whether they are under relaxed or stringent control.

More than 30 different replicons have been identified in plasmids. However, almost all plasmids used routinely in molecular cloning carry a replicon derived from pMB1. pUC plasmids contain a modified pMB1 replicon, have relaxed control, and replicate to a very high copy number, otherwise pSC101 has stringent control and maintain low-copy number. Generally, high-copy number plasmid will result in higher yield.

Very large plasmids are often maintained at very low copy numbers per cell.

GeneAll® Hybrid-Q™ Plasmid Rapidprep Kit Procedure is optimized to high-copy number plasmid, so more starting sample may be needed if low-copy number plasmids are used.

Table 1. Replicons carried by various plasmid vectors

Plasmid	Size in bp	Copy number	Replicon
pUC series	2,686	500~700	pMB1
pBluescript series	~3,000	300~500	ColE1
pGEM series	~3,000	300~400	pMB1
pMK16 and derivatives	~4,500	> 15	ColE1
pBR322 and derivatives	4,362	15~20	pMB1
pACYC and derivatives	~4,000	18~22	p15A
pSC101 and derivatives	9,263	~5	pSC101
pRK353 and derivatives	~11,100	~15	R6K

Most *E.coli* strains can be used to propagate and isolate plasmid DNA. Host strains such as DH5 α and XL-1 blue yield DNA of very high-quality. But some strains, particularly those derived from HB101 (e.g. TG1 and the JM series), release relatively large amount of carbohydrates when they are lysed. Carbohydrates can inhibit the activity of many restriction enzymes and polymerases, if not completely removed. Many *endA*⁺ strains produce endonuclease I which is encoded in *endA* and cleaves double-strand DNA (See page 12). If endonuclease I is not completely removed during plasmid preparations, the plasmid DNA in eluate is degraded during subsequent incubation in the presence of Mg²⁺ (e.g. during PCR or the incubation with restriction enzyme). This problem can be avoided by use of *endA*⁻ strains (denoted as *endA1*) such as DH5 α and XL1Blue. Extra wash with buffer AW will also help prevent the degradation of DNA.

GeneAll[®] Hybrid-Q[™] Plasmid Rapidprep Kit is optimized to Luria-Bertani (LB) broth which is the most widely used culture medium for propagation of *E.coli*. Use of other rich broth such as Terrific Broth (TB) or 2xYT will lead to very high cell density. If these media are used, starting sample volume should be reduced not to overload GeneAll[®] spin column and buffer system. Otherwise, the volume of buffer S1, S2 and G3 should be increased for efficient lysis. Overnight culture in TB or 2xYT may yield 2~5 times the number of cells compared to cultures grown in LB broth. TB or 2xYT can be used to obtain more yield of plasmid DNA, in case of low-copy number plasmid.

■ Alkaline lysis

Harvested bacterial culture is resuspended by buffer S1 in the presence of RNase A. Exposure of bacterial suspensions to the strongly anionic detergent at high pH (Buffer S2, SDS/NaOH) opens the cell wall, denatures chromosomal DNA and proteins, and releases plasmid DNA into the supernatant. Although buffer S2, the alkaline solution, completely disrupts base pairing, the strands of closed circular plasmid DNA are unable to separate from each other because they are topologically intertwined.

As long as the intensity and duration of exposure to high pH (OH^-) is not too great, the two strands of plasmid DNA fall once again into register when the pH is returned to neutral. However, prolonged exposure to denaturing condition causes closed circular DNA to enter an irreversibly denatured state. The resulting collapsed coil, which can not be cleaved with restriction enzymes, migrates through agarose gels at about twice the rate of native superhelical closed circular DNA and stains poorly with intercalating dyes.

During lysis, bacterial proteins, broken cell walls, and denatured chromosomal DNA become enmeshed in large complexes that are coated with dodecyl sulfate. These complexes are efficiently precipitated from solution by addition of buffer G3 which replaces sodium ions by potassium ions and adjusts the lysate to high-salt binding conditions.

Vigorous handling of lysate may cause the denatured chromosomal DNA to shear, followed by contamination of genomic DNA. It is important for good result that the solution is gently but thoroughly mixed to ensure complete precipitation.

■ Filtration of lysate with EzClear™ Filter Column

After mixing with buffer G3, the cellular debris and precipitates should be removed completely not to clog GeneAll® spin column in subsequent binding. New patented EzClear™ filter column facilitates the clearance of the lysate by filtration instead of tedious centrifugation which has been used widely in traditional methods.

In the rapid protocol, EzClear™ filter column is assembled with GeneAll® spin column, and this column stack makes it one-step the clearance of lysate and the binding of plasmid DNA to spin column membrane.

■ Washing

When working with *endA*⁺ strains, endonucleases can be efficiently removed by optional wash step with buffer AW to ensure that plasmid DNA is not degraded during storage or enzyme reactions.

Because buffer AW enhances the quality of plasmid DNA by removal of residual proteins, it is also recommended when working with low-copy plasmids which are generally used with larger culture volume. Buffer PW removes salts and other cellular components bound nonspecifically to column membrane.

Table 2. The genotype of various *E.coli* strains

<i>EndA</i>⁺ strains	<i>EndA</i> strains
BL21(DE3), CJ236, HB101, JM83, JM101, JM110, LE392, MC1061, NM series, P2392 PR series, RRI, TBI, TGI, BMH71-18, ESI301, wild-type and etc.	DH1, DH20, DH21, DH5α, JM103, JM105, JM106, JM107, JM108, JM109, MM294, SK1590, SRB, XLI-Blue, XLO and etc.

■ Elution

Purified DNA can be eluted in low salt buffer or deionized water depending on the need for downstream applications. Buffer EB contains 10mM TrisCl, pH8.5. When using water for eluent, make sure that the pH value is within 7.0 and 8.5.

Because plasmid in water is susceptible to hydrolysis and water lacks a buffering agent, it is recommended to store below -20°C. The elution volume can be adjusted as necessity, but it has to be over the minimum requirement to soak completely the spin column membrane. To get high concentration of DNA, decrease the volume of elution buffer to minimum. For higher yield, increase the volume of elution buffer and repeat the elution step again. The concentration and yield as the change of elution volume is shown below.

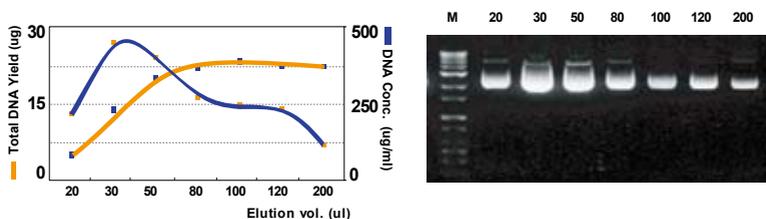


Figure 1. The overall yield and concentration of plasmid DNA depending on the volume of elution. pUC18 plasmid DNA was purified from 3 ml of overnight cultured DH5 α using GeneAII[®] Hybrid-Q[™] Plasmid Rapidprep Kit. Plasmid DNA was eluted with the indicated volume of buffer EB, and resolved on 1 % agarose gel.

Hybrid-Q™

Rapid Protocol

1. Pellet 1~3 ml of culture by centrifugation
2. Resuspend in 170 ul of buffer S1
3. Add 170 ul of buffer S2 and mix by inverting
4. Add 250 ul of buffer G3 and mix by inverting
5. Transfer the lysate to EzClear™ column stack by decanting
6. Centrifuge for 30 sec and discard the EzClear™ filter (upper, violet)
7. (Optional) Add 500 ul of buffer AW and centrifuge for 30 sec
8. Add 700 ul of buffer PW and centrifuge for 30 sec
9. Centrifuge for additional 1 min
10. Apply 50 ul of buffer EB and centrifuge for 1 min

GeneAll® Hybrid-Q™ Rapid Protocol

Before experiment

Unless there is another indication, all centrifugation steps should be performed at full speed ($> 10,000 \text{ xg}$ or $10,000\sim 14,000 \text{ rpm}$) in a microcentrifuge at room temperature.

Add all of RNase solution into buffer S1 before first use and store it at 4°C

Buffer S2 and G3 may precipitate at cool ambient conditions. If precipitate appears, dissolve it in 37°C water bath until completely dissolved.

Prepare new 1.5 ml or 2 ml tubes.

1. Pellet 1~3 ml of the bacterial culture by centrifugation for 1 min at 13,000 xg in a microcentrifuge. Discard the supernatant as much as possible.

Use the appropriate volume of bacterial cultures; too much amount of starting sample can clog the EzClear™ filter. Bacterial culture should be grown for 16 to 21 hours in LB media containing a selective antibiotic. Use of other rich broth, such as TB or 2xYT, and/or higher culture volume can cause reduction of lysis efficiency, clogging of EzClear™ filter column or overload of a spin column, resulting in unsatisfactory yield. For more than 2 ml of overnight culture, bacterial cells can be collected in 15 ml conical tube by centrifugation for 5 min at $10,000 \text{ xg}$ in a tabletop centrifuge. Alternatively, bacterial cells can be collected repeatedly in 1.5 ml or 2 ml microcentrifuge tube.

2. Resuspend pelleted bacterial cells thoroughly in 170 ul of Buffer S1.

It is essential to thoroughly resuspend the cell pellet.

* Add RNase solution into buffer S1 before the first use

3. Add 170 ul of Buffer S2 and mix by inverting the tube 3~4 times (DO NOT VORTEX).

Incubate until the cell suspension becomes clear and viscous, but DO NOT incubate for more than 5 min. It is important to proceed to next step immediately after the lysate becomes clear without any cloudy clumps.

If precipitated material has formed in buffer S2, heat to dissolve at 37°C. Precipitated buffer S2 may cause significant decrease in DNA recover yield.

4. Add 250 ul of Buffer G3 and immediately mix by inverting the tube 4~5 times (DO NOT VORTEX).

For better precipitation, mix the lysate gently but completely and immediately after addition of buffer G3. Vigorous handling may lead to the contamination of genomic DNA and the decrease in quality of plasmid DNA.

5. Transfer carefully all of the lysate to EzClear™ Column stack by decanting or pipetting. Centrifuge for 30~60 sec. Discard the upper EzClear™ Filter Column unit, remove the spin column, discard the pass-through fraction, and re-insert the spin column to the collection tube.

It may be necessary to use “Wide-bore Tip” or to cut the end off the pipet tip to transfer the lysate to the EzClear™ filter column by pipetting. However, decanting directly to EzClear™ filter unit may be handy method for transferring.

A little residual liquid can remain in the upper EzClear™ filter column. But this will not affect DNA recovery.

- 6. (Optional :) Apply 500 ul of Buffer AW and centrifuge for 30 sec. Remove the spin column, discard the pass-through, and re-insert the spin column to the collection tube.**

This step is necessary to remove any trace of nuclease activity from *endA*⁺ strain.

The wildtype and some *E.coli* strains produce endonuclease I which is encoded in gene *endA* and degrades double-stranded DNA. The *E.coli* genotype *endA1* refers to a mutation in the wildtype *endA* gene, which produces an inactive form of the nuclease. *E.coli* strains with this mutation are referred to as *endA*⁻. The absence of *endA1* in the genotype-list denotes the presence of the wildtype gene, which expressed an active endonuclease I. The wildtype is indicated as *endA*⁺. The genotype of several *E.coli* strains is shown in table 2 at page 12.

When the low-copy-plasmid is used, it is recommended to carry out this step, even though *endA*⁻ strains.

- 7. Apply 700 ul of Buffer PW and centrifuge for 30 sec. Remove the spin column, discard the pass-through, and re-insert the spin column to the collection tube.**
- 8. Centrifuge for an additional 1 min to remove residual wash buffer. Transfer the spin column to a new 1.5 ml tube (Not provided).**

If carryover of buffer PW occurs, centrifuge again for 1 min before proceeding to next step. Residual ethanol from buffer PW may interfere with the subsequent reactions.

9. Add 50 ul of Buffer EB or deionized distilled water, let stand for 1 min, and centrifuge for 1 min.

Ensure that buffer EB or distilled water is dispensed directly onto the center of spin column membrane for optimal elution of DNA.

Elution volume can be increased to 100~200 ul and it will increase the total yields of plasmid but decrease the concentration of eluate. For higher concentration of eluate, elution volume can be decreased to 40 ul minimum.

For long-term storage, eluting in buffer EB (10mM TrisCl, pH 8.5) and storing below -20°C is recommended. When using water for elution, ensure that the pH value of water is between 7.0 and 8.5.

Some larger plasmids (> 10 kb) usually may not be eluted optimally unless pre-heated (70°C) buffer EB or ddH₂O is applied for elution. Incubate for 2 min after addition of pre-heated elution buffer.

Hybrid-Q™

Standard Protocol

1. Pellet up to 5 ml of culture by centrifugation
2. Resuspend in 250 ul of buffer S1
3. Add 250 ul of buffer S2 and mix by inverting
4. Add 350 ul of buffer G3 and mix by inverting
5. Centrifuge for 10 min
6. Transfer the cleared lysate to spin column and centrifuge for 30 sec
7. (Optional) Add 500 ul of buffer AW and centrifuge for 30 sec
8. Add 700 ul of buffer PW and centrifuge for 30 sec
9. Centrifuge for additional 1 min
10. Apply 50 ul of buffer EB and centrifuge for 1 min

GeneAll® Hybrid-Q™ Standard Protocol

Before experiment

Unless there is another indication, all centrifugation steps should be performed at full speed (> 10,000 xg or 10,000~14,000 rpm) in a microcentrifuge at room temperature.

Add all of RNase solution into buffer S1 before first use and store it at 4 °C

Buffer S2 and G3 may precipitate at cool ambient conditions. If precipitate appears, dissolve it in 37 °C water bath until completely dissolved.

Prepare new 1.5 ml or 2 ml tubes.

- 1. Pellet up to 5 ml of the bacterial culture by centrifugation for 5 min at 10,000 xg in a tabletop centrifuge. Discard the supernatant as much as possible.**

Use the appropriate volume of bacterial cultures; a. Excessive sample can not be lysed efficiently and it can lead to poor result. b. Because of the column binding capacity, the large sample does not produce much yield proportionally. Bacterial culture should be grown for 16 to 21 hours in LB media containing a selective antibiotic. Use of other rich broth, such as TB or 2xYT, and/or higher culture volume can cause reduction of lysis efficiency or overload of a spin column, resulting in unsatisfactory yields.

Alternatively, bacterial cells can be pelleted repeatedly in 1.5 ml or 2 ml microcentrifuge tube by centrifugation for 1 min at full speed.

- 2. Resuspend pelleted bacterial cells thoroughly in 250 ul of Buffer S1. Transfer the suspension to a new 1.5 ml tube.**

It is essential to thoroughly resuspend the cell pellet.

* Add RNase solution into buffer S1 before first use.

3. Add 250 ul of Buffer S2 and mix by inverting the tube 4 times (DO NOT VORTEX).

Incubate until the cell suspension becomes clear and viscous, but DO NOT incubate for more than 5 min. It is important to proceed to next step immediately after the lysate becomes clear without any cloudy clumps.

If precipitated material has formed in buffer S2 before use, heat to dissolve at 37°C. Precipitated buffer S2 may cause significant decrease in DNA recover yield.

4. Add 350 ul of Buffer G3 and immediately mix by inverting the tube 4-6 times (DO NOT VORTEX).

For better precipitation, mix the lysate gently but completely and immediately after addition of buffer G3.

5. Centrifuge for 10 min.

6. Transfer carefully the supernatant to a spin column by decanting or pipetting. Centrifuge for 30 sec. Remove the spin column, discard the pass-through, and re-insert the spin column to the collection tube.

Avoid the white precipitate cotransferring into the spin column.

- 7. (Optional :) Apply 500 ul of Buffer AW and centrifuge for 30 sec. Remove the spin column, discard the pass-through, and reinsert the spin column to the collection tube.**

This step is necessary to remove any trace of nuclease activity from *endA*⁺ strain. The wildtype and some *E.coli* strains produce endonuclease I which is encoded in gene *endA* and degrades double-stranded DNA.

The *E.coli* genotype *endA1* refers to a mutation in the wildtype *endA* gene, which produces an inactive form of the nuclease. *E.coli* strains with this mutation are referred to as *endA*⁻.

The absence of *endA1* in the genotype-list denotes the presence of the wildtype gene, which expressed an active endonuclease I. The wildtype is indicated as *endA*⁺. The genotype of several *E.coli* strains is shown in table 2 at page 12.

When low-copy-plasmid is used, it is recommended to carry out this step, even though *endA*⁻ strains.

- 8. Apply 700 ul of Buffer PW and centrifuge for 30 sec. Remove the spin column, discard the pass-through, and re-insert the spin column to the collection tube.**

- 9. Centrifuge for an additional 1 min to remove residual wash buffer. Transfer the spin column to a new 1.5 ml tube (Not provided).**

This step removes residual ethanol from spin column membrane. Residual ethanol in eluate may inhibit subsequent enzymatic reaction. If carryover of buffer PW occurs, centrifuge again for 1 min before proceeding to next step.

10. Add 50 ul of Buffer EB or deionized distilled water, let stand for 1 min, and centrifuge for 1 min.

Ensure that buffer EB or distilled water is dispensed directly onto the center of spin column membrane for optimal elution of DNA. Elution volume can be increased to 200 ul maximum and it will increase the total yield of plasmid but decrease the concentration of eluate.

For long-term storage, eluting in buffer EB (10mM TrisCl, pH 8.5) and storing below -20°C is recommended. When using water for elution, ensure that the pH of water is within the range of 7.0 ~ 8.5.

Some larger plasmids (> 10 kb) usually may not be eluted optimally unless pre-heated (70°C) buffer EB or ddH₂O is applied for elution. Incubate for 2 min after addition of pre-heated elution buffer.

Hybrid-Q™

Low-copy plasmid Protocol

1. Pellet up to 10 ml of culture into 2 ml tube by centrifugation
2. Resuspend in 400 ul of buffer S1
3. Add 400 ul of buffer S2 and mix by inverting
4. Add 600 ul of buffer G3 and mix by inverting
5. Centrifuge for 10 min
6. Transfer 700 ul of the cleared lysate to spin column and centrifuge for 30 sec
7. Transfer the remainder into spin column and centrifuge for 30 sec
8. Add 500 ul of buffer AW and centrifuge for 30 sec
9. Add 700 ul of buffer PW and centrifuge for 30 sec
10. Centrifuge for additional 1 min
11. Apply 50 ul of buffer EB and centrifuge for 1 min

GeneAll® Hybrid-Q™

Low-copy plasmid Protocol

Before experiment

Unless there is another indication, all centrifugation steps should be performed at full speed (> 10,000 xg or 10,000~14,000 rpm) in a microcentrifuge at room temperature.

Add all of RNase solution into buffer S1 before first use and store it at 4 °C

Buffer S2 and G3 may precipitate at cool ambient conditions. If precipitate appears, dissolve it in 37 °C water bath until completely dissolved.

Prepare new 1.5 ml or 2 ml tubes.

Due to the need of additional buffer for this protocol, fewer preparations can be carried out. Buffers can be purchased separately as accessory.

- 1. Pellet up to 10 ml of the bacterial culture by centrifugation for 5 min at 10,000 xg in a tabletop centrifuge. Discard the supernatant as much as possible.**

Use the appropriate volume of bacterial cultures. Excessive sample can not be lysed efficiently and it can lead to poor result.

Bacterial culture should be grown for 16 to 21 hours in LB media containing a selective antibiotic. Use of other rich broth, such as TB or 2xYT, and/or higher culture volume can cause reduction of lysis efficiency, resulting in unsatisfactory yields.

Alternatively, bacterial cells can be pelleted repeatedly into a 2 ml microcentrifuge tube by centrifugation for 1 min at full speed.

- 2. Resuspend pelleted bacterial cells thoroughly in 400 ul of Buffer S1. Transfer the suspension to a new 2 ml tube.**

It is essential to thoroughly resuspend the cell pellet.

* Add RNase solution into buffer S1 before first use.

3. Add 400 ul of Buffer S2 and mix by inverting the tube 4 times (DO NOT VORTEX).

Incubate until the cell suspension becomes clear and viscous, but DO NOT incubate for more than 5 min. It is important to proceed to next step immediately after the lysate becomes clear without any cloudy clumps.

If precipitated material has formed in buffer S2 before use, heat to dissolve at 37°C. Precipitated buffer S2 may cause significant decrease in DNA recover yield.

4. Add 600 ul of Buffer G3 and immediately mix by inverting the tube 4-6 times (DO NOT VORTEX).

For better precipitation, mix the lysate gently but completely and immediately after addition of buffer G3.

5. Centrifuge for 10 min. Transfer carefully the supernatant to a new 2 ml tube by decanting or pipetting.

Avoid the white precipitate cotransferring into a new tube.

6. Transfer 700 ul of the cleared lysate into a spin column. Centrifuge for 30 sec. Remove the spin column, discard the pass-through, and re-insert the spin column to the collection tube.

7. Repeat the step 6 with the remaining cleared lysate.

8. Apply 500 ul of Buffer AW and centrifuge for 30 sec. Remove the spin column, discard the pass-through, and reinsert the spin column to the collection tube.

This step is necessary to remove any trace of nuclease activity from *endA*⁺ strain. The wildtype and some *E.coli* strains produce endonuclease I which is encoded in gene *endA* and degrades double-stranded DNA.

The *E.coli* genotype *endA1* refers to a mutation in the wildtype *endA* gene, which produces an inactive form of the nuclease. *E.coli* strains with this mutation are referred to as *endA*⁻.

The absence of *endA1* in the genotype-list denotes the presence of the wildtype gene, which expressed an active endonuclease I. The wildtype is indicated as *endA*⁺. The genotype of several *E.coli* strains is shown in table 2 at page 12.

When low-copy-plasmid is used, it is recommended to carry out this step, even though *endA*⁻ strains.

9. Apply 700 ul of Buffer PW and centrifuge for 30 sec. Remove the spin column, discard the pass-through, and re-insert the spin column to the collection tube.

10. Centrifuge for an additional 1 min to remove residual wash buffer. Transfer the spin column to a new 1.5 ml tube (Not provided).

This step removes residual ethanol from spin column membrane. Residual ethanol in eluate may inhibit subsequent enzymatic reaction. If carryover of buffer PW occurs, centrifuge again for 1 min before proceeding to next step.

11. Add 50 ul of Buffer EB or deionized distilled water, let stand for 1 min, and centrifuge for 1 min.

Ensure that buffer EB or distilled water is dispensed directly onto the center of spin column membrane for optimal elution of DNA. Elution volume can be increased to 200 ul maximum and it will increase the total yield of plasmid but decrease the concentration of eluate.

For long-term storage, eluting in buffer EB (10mM TrisCl, pH 8.5) and storing below -20°C is recommended. When using water for elution, ensure that the pH of water is within the range of 7.0 ~ 8.5.

Some larger plasmids (> 10 kb) usually may not be eluted optimally unless pre-heated (70°C) buffer EB or ddH₂O is applied for elution. Incubate for 2 min after addition of pre-heated elution buffer.

Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low or no yield of plasmid DNA	Poor lysis due to too many cells in the sample.	Cultures should be grown for 16~21 hours in proper media with antibiotics. Reduce the volume of sample.
	Low-copy-number plasmid used	Low-copy-number plasmid may yield as little as 0.5 ug of DNA from a 5 ml overnight culture. Increase the culture volume or use high-copy-number plasmid or rich broth, if possible.
	Poor resuspension of bacterial pellets in Buffer S1	Bacterial cell pellets should be thoroughly resuspended in buffer S1.
	Buffer S2 precipitated	Redissolve buffer S2 by warming at 37°C or above.
	Insufficient digestion with RNase	Excess RNA can interfere the binding of plasmid DNA with GeneAll® spin column membrane. Store buffer S1 at 4°C after the addition of RNase. If buffer S1 containing RNase is more than a year old, the activity of RNase can be decreased.
Low purity	Inadequate elution Buffer	DNA can be eluted only in low salt condition. Buffer EB (10mM TrisCl, pH 8.5) has the optimal elution efficiency, but other elution buffer can be engaged as user's need. Elution efficiency is dependent on pH and the maximum efficiency is achieved between 7.0 and 8.5. When using water for elution, make sure the pH value.
	Contamination of precipitate when binding	When the cleared lysate is transferred to GeneAll® spin column, ensure that any precipitate does not contain to the transfer.

Facts	Possible Causes	Suggestions
Chromosomal DNA contamination	Mis-handling of the lysate after addition of Buffer G3	<p>Vigorous vortexing after addition of buffer G3 can cause shearing of chromosomal DNA followed by chromosomal DNA contamination.</p> <p>Handle gently the lysate after addition of buffer G3. Simple inverting and rotating tube to cover walls with lysate is sufficient for mixing.</p>
	Too large sample	<p>Reduce the sample volumes.</p>
Smearing of plasmid DNA	Too long lysis time	<p>Too long lysis under buffer S2 can cause chromosomal DNA contamination. Proceed to next step immediately after no more clumps are visible in the lysate. Lysis time should not be over 5 min in any case.</p>
	Vigorous mixing in Buffer S2	<p>Vigorous handling after addition of buffer S2 can lead to irreversible denaturation of plasmid DNA. Gentle inverting and rotating tube to cover walls with viscous lysate is sufficient for mixing.</p>
EzClear™ filter clogging	Too many cells in the sample	<p>Reduce the sample volume.</p>
RNA Contamination	RNase omitted or old	<p>RNase solution should be added to buffer S1 before first use. If buffer S1 containing RNase is more than a year old, the activity of RNase can be decreased. Add additional RNase (working concentration = 100 ug/ml). Buffer S1 containing RNase should be stored at 4 °C.</p>
	Too many cells in sample	<p>Reduce the sample volume. Too many cells may not be subjected properly to RNase digestion.</p>

Facts	Possible Causes	Suggestions
High salt concentration in eluate	Improper wash step	Ensure that washing steps are performed properly. Alternatively, incubate for 5 min at room temperature after applying buffer PW in wash step.
Plasmid DNA degradation	Nuclease contamination	For <i>endA</i> ⁺ strains such as HB101 and the JM series, washing with buffer AW should be carried out properly. Refer to page 12
DNA floats out of well while loading of agarose gel	Ethanol is not completely removed during wash steps	Ensure that washing steps are performed properly. GeneAll® spin column membrane should be completely dried via additional centrifugation or air-drying for good result.
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.
	Residual ethanol in eluate	Ensure that the washing steps are performed properly. GeneAll® spin column membrane should be completely dried via additional centrifugation or air-drying.

Ordering Information

Products	Size	Type	Cat. No.
GeneAll® Hybrid-Q™ for rapid preparation of plasmid DNA			
Plasmid Rapidprep	50	spin	100-150
	200		100-102
GeneAll® Exprep™ for preparation of plasmid DNA			
Plasmid SV mini	50	spin / vacuum	101-150
	200		101-102
	1,000		101-111
Plasmid SV Midi**	26	spin / vacuum	101-226
	50		101-250
	100		101-201
Plasmid SV Quick	50	mini / spin	101-050
	200		101-002
	1,000		101-011
GeneAll® Exfection™ for preparation of highly pure plasmid DNA			
Plasmid LE mini (Low Endotoxin)	50	spin / vacuum	111-150
	200		111-102
Plasmid LE Midi* (Low Endotoxin)	26	spin / vacuum	111-226
	100		111-201
Plasmid EF Midi* (Endotoxin Free)	20	spin	121-220
	100		121-201
GeneAll® Expin™ for purification of fragment DNA			
Gel SV	50	mini / spin / vacuum	102-150
	200		102-102
PCR SV	50	mini / spin / vacuum	103-150
	200		103-102
CleanUp SV	50	mini / spin / vacuum	113-150
	200		113-102
Combo GP	50	mini / spin / vacuum	112-150
	200		112-102
GeneAll® Exgene™ for isolation of total DNA			
Tissue SV mini (plus!)*	100	spin / vacuum	104(9)-101
	250		104(9)-152
Tissue SV Midi (plus!)**	26	spin / vacuum	104(9)-226
	100		104(9)-201
Tissue SV MAXI (plus!)**	10	spin / vacuum	104(9)-310
	26		104(9)-326
Blood SV mini	100	spin / vacuum	105-101
	250		105-152
Blood SV Midi**	26	spin / vacuum	105-226
	100		105-201
Blood SV MAXI**	10	spin / vacuum	105-310
	26		105-326
Cell SV mini	100	spin / vacuum	106-101
	250		106-152
Cell SV MAXI**	10	spin / vacuum	106-310
	26		106-326
Clinic SV mini	100	spin / vacuum	108-101
	250		108-152

Products	Size	Type	Cat. No.
GeneAll® Exgene™ for isolation of total DNA			
Clinic SV Midi	26	spin / vacuum	108-226
	100		108-201
Clinic SV MAXI**	10	spin / vacuum	108-310
	26		108-326
Plant SV mini	100	spin / vacuum	117-101
	250		117-152
Plant SV Midi**	26	spin / vacuum	117-226
	100		117-201
Plant SV MAXI**	10	spin / vacuum	117-310
	26		117-326
GMO SV mini	50	spin / vacuum	107-150
	200		107-102
GeneAll® GenEx™ for isolation of total DNA			
Genomic Sx [†]	100	mini / solution	208-001
Genomic Sx [†]	500	mini / solution	208-005
Genomic Lx ^{††}	100	MAXI / solution	208-301
GeneAll® RiboEx™ for preparation of total RNA			
RiboEx™	100	solution	301-001
	200		301-002
RiboEx™_column	50	spin	301-150
	100		302-001
RiboEx™ LS	200	solution	302-002
	100		302-002
Ribo_clear™	50	spin	303-150
Ribo_spin vRD™	50	spin	302-150
GeneAll® AmpONE™ for PCR amplification			
Taq DNA polymerase	250 U	(2.5 U/μℓ)	501-025
	500 U		501-050
	1,000 U		501-100
α-Taq DNA polymerase	250 U	(2.5 U/μℓ)	502-025
	500 U		502-050
	1,000 U		502-100
Pfu DNA polymerase	250 U	(2.5 U/μℓ)	503-025
	500 U		503-050
	1,000 U		503-100
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	96 tubes	521-200
	50 μℓ		521-500
α-Taq Premix	20 μℓ	96 tubes	522-200
	50 μℓ		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

* 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.

†† On the basis of DNA purification from 10 ml whole blood.

Note.



GeneAll

GENEALL BIOTECHNOLOGY CO., LTD

www.geneall.com

Banseok Bld., 128 Oguem-dong,
Songpa-gu, Seoul, KOREA 138-859

E-MAIL sales@geneall.com

T E L 82-2-407-0096

F A X 82-2-407-0779

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