Ver 1.1

Handbook for

PLASMID LE MINI
PLASMID LE MIDI
PLASMID EF MIDI

exfection™

Exfection™ Plasmid Kits Protocol Handbook

For purification of advanced transfection-grade plasmid DNA

GeneAll

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.) : bnp@geneall.com

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This protocol handbook is included in :

GeneAll® Exfection™ Plasmid LE mini (111-150, 111-102)
GeneAll® Exfection™ Plasmid LE Midi (111-226, 111-201)
GeneAll® Exfection™ Plasmid EF Midi (121-220)

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

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Kit Contents

	Exfection™ LE mini		Exfection™ LE Midi	
Cat. No.	**111-150**	**111-102**	**111-226**	**111-201**
Size	**mini**	**mini**	**Midi**	**Midi**
No. of preparation	50	200	26	100
GeneAll® SV Column type Qe	50	200	-	-
GeneAll® SV Column type E	-	-	26	100
EzClear™ Filter Column	-	-	26	100
Collection Tube	50	200	78	300
Buffer P1	20 ml	60 ml	80 ml	300 ml
Buffer P2	20 ml	60 ml	80 ml	300 ml
Buffer G3	25 ml	90 ml	110 ml	400 ml
Buffer EW1	40 ml	150 ml	300 ml	1000 ml
Buffer EW2	60 ml	250 ml	400 ml	1500 ml
Buffer EF*	15 ml	30 ml	60 ml	180 ml
RNase A	2 mg	6 mg	8 mg	30 mg
Protocol Handbook	1	1	1	1

	Exfection™ EF Midi
Cat. No.	**121-220**
Size	**Midi**
No. of preparation	20
GeneAll® SV Column type E	20
EzClear™ Filter Column	20
15 ml Collection Tube	20
50 ml Collection Tube	60
Buffer P1	100 ml
Buffer P2	100 ml
Buffer P3	100 ml
Buffer ER	15 ml
Buffer EG	150 ml
Buffer EW1	250 ml
Buffer EW2	350 ml
Buffer EF*	60 ml
RNase A	10 mg
Protocol Handbook	1

** 10mM TrisCl, pH 8.5, endotoxin-free*

Precautions and Disclaimer

GeneAll® Exfection™ Plasmid Kits are 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 the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Chemical Hazard

The buffers included in GeneAll® Exfection™ kits contain 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.

Some buffers including G3 and EG contain chaotropic salts. It can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste.

Quality Control

All components in GeneAll® Exfection™ plasmid kits are manufactured in strictly clean condition, and its degree of cleanness is monitored periodically. Restriction enzyme assay, gene cloning, PCR amplification assay, automated sequencing analysis and LAL test as quality control are carried out from lot to lot thoroughly, and only the qualified is delivered.

Storage condition

GeneAll® Exfection™ kits are shipped at room temperature. All components are stable at room temperature until the date of expiration that is printed on the product label. After the addition of RNase A, Buffer P1 is stable for 1 year when stored at 4°C.

In cold ambient conditions, buffer P2 and G3 may exhibit salt precipitation and this causes reduction of DNA recover-yields. If so, heat the bottle with occasional swirling in 37°C water bath until completely dissolved.

Buffer P3 can be stored at 4°C. Pre-chilled buffer P3 may lead to better result.

Product Specifications

	Exfection™ LE mini	Exfection™ LE Midi*	Exfection™ EF Midi*
Format	Spin/Vacuum	Spin/Vacuum	Spin
Recommended sample volume	5 ml	50 ml	100 ml
Maximum sample volume	10 ml	100 ml	150 ml
Clearing of lysate	Centrifuge	EzClear™	EzClear™
Preparation time	< 30 min	< 50 min	< 70 min
Maximum loading volume	800 ul	15 ml	15 ml
Binding capacity	30 ug	300 ug	300 ug
The level of endotoxins	< 10 EU/ug	< 10 EU/ug	< 0.1 EU/ug
Recovery	80 ~ 95 %	85 ~ 95 %	75 ~ 90 %
Minimum elution volume	50 ul	500 ul	500 ul

** GeneAll® Exfection™ Midi procedure requires a conventional centrifuge which has a swinging-out bucket and ability of 4,000 ~ 5,000 xg.*

GeneAll®

Exfection™ LE and Exfection™ EF Plasmid DNA Purification Kit

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Plasmid DNA Purification Kit Exfection™ LE and Exfection™ EF Plasm

Introduction

GeneAll® Exfection™ plasmid kits provide a simple and fast method for the purification of plasmid DNA with low endotoxin contaminants. Endotoxins (also known as lipopolysaccharides, LPS) are present in the cell membranes of gram-negative bacteria, such as *Escherichia coli*. It is a common contaminant in plasmid preparations and can significantly reduce transfection efficiencies, if not removed during DNA preparations. Endotoxins can be removed with Exfection™ procedures by two major technologies: advanced phase separation and endotoxin removal washing. The contamination of endotoxin can be reduced to 0.1 EU/ug with Exfection™ EF plasmid kit. Exfection™ LE plasmid kit uses a new developed wash-buffer which can remove both endotoxins and endonucleases, and this kit can be used for the transfection of most cell-lines.

These kits can be used to isolate and purify any plasmid, but works most efficiently when the plasmid is less than 20,000 bp in size. All process to prepare pure plasmid DNA takes less time than other anion-exchange resin based-kit and simultaneous processing of multiple samples can be easily performed. Up to 30 ug (300 ug for Midi) of supercoiled plasmid can be purified using Exfection™ plasmid kit and this pure plasmid DNA is ready for PCR, cloning, fluorescent sequencing, synthesis of labeled hybridization probes, cell transfection, electroporation, enzymatic restriction analysis, and other sensitive applications without further manipulation.

Principle of Method

GeneAll® Exfection™ Plasmid kits utilize glass microfiber membrane based on the advanced 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 centrifugation for mini kit and by EzClear™ Filter Column for Midi kits. Endotoxins are removed by advanced phase separation technology.

In the presence of high salt, plasmid DNA in cleared lysate binds selectively to glass microfiber membrane in GeneAll® Exfection™ spin column. Bound plasmid DNA is purified in a series of washing steps to eliminate contamination of other bacterial components, such as endotoxins and endonuclease. Finally elution by low salt buffer or deionized water releases plasmid DNA from the glass microfiber membrane. This simple method not only eliminates the need for organic solvent extractions and alcohol precipitation, but also reduce the experiment time of tedious cesium-chloride method or anion-exchange chromatography.

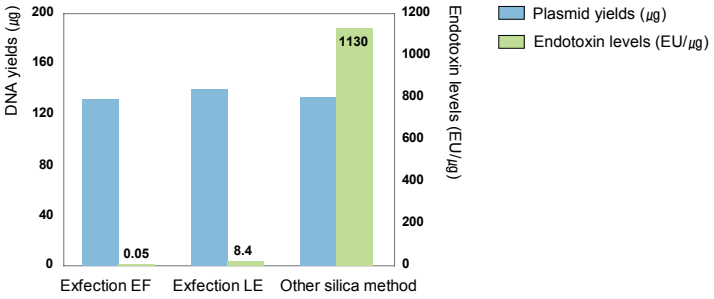
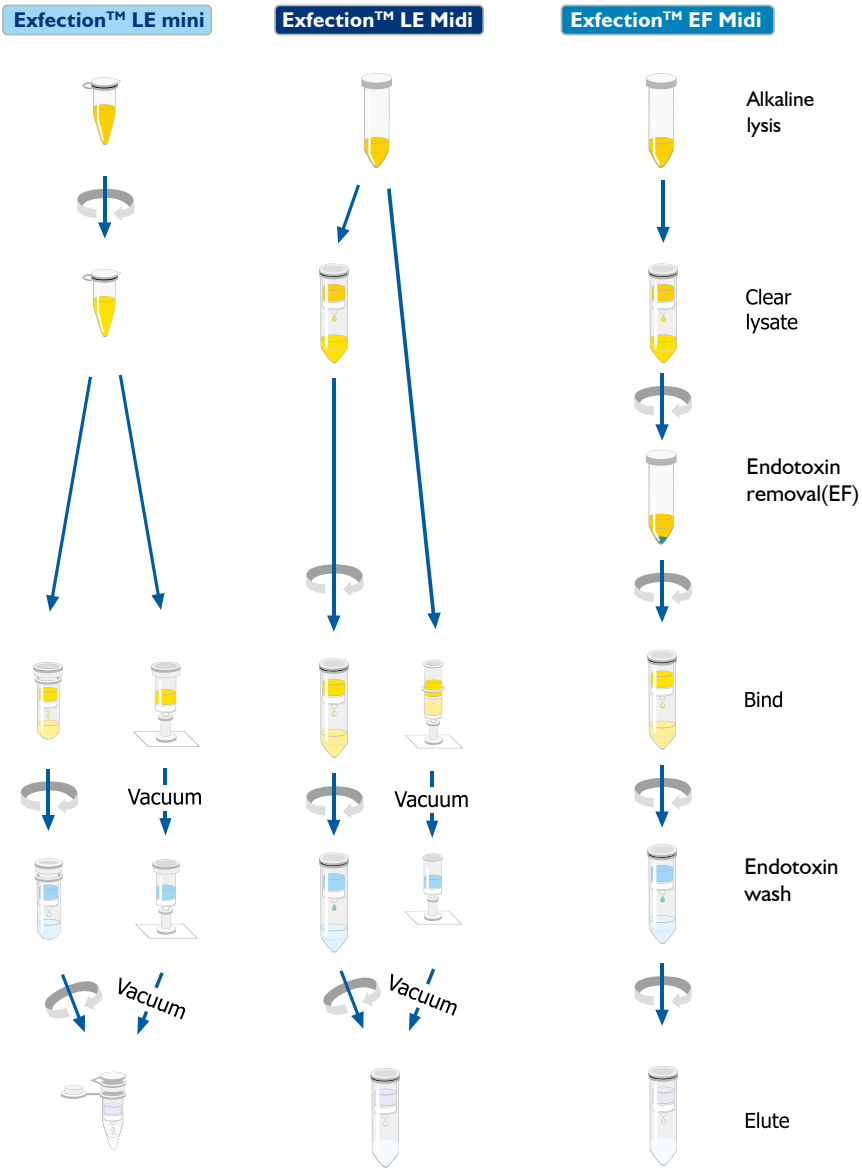


Fig. 1 Comparison of average yields and endotoxin levels in pEGFP-N3 prepared each from 50 ml overnight culture of DH10b by the methods indicated. Endotoxin levels were reduced by more than 10,000 fold using Exfection™ EF procedure, compared to other silica-based kit.

GeneAll® Exfection™ Procedures



General considerations

Starting materials

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.

Wherever 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,

Table I. 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

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® Plasmid Kit Procedure is optimized to high-copy number plasmid, so larger starting sample may be needed if low-copy number plasmids are used.

Most *E.coli* strains can be used to propagate and isolate plasmid DNA. Host strains such as DH5α and XL-1Blue 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. If endonuclease I is not completely removed during DNA preparations, the plasmid DNA in eluate is

degraded during subsequent incubation in the presence of Mg^{2+} (e.g. during incubation with restriction enzyme). The wash with buffer EW1 efficiently removes endonucleases from column membrane. Otherwise, this problem can be avoided by use of *endA*$^-$ strains (denoted as *endA*1) such as DH5α and XL1Blue.

GeneAll® Plasmid Kit Series is optimized to Luria-Bertani (LB) broth which is the most widely used culture medium for propagation of *E.coli.* Use of 5~10 ml LB culture for mini or 50~150 ml LB culture for Midi generally results in good plasmid yields and endotoxin levels. However, the optimal volume of culture to use depends upon the strain, the plasmid, and the density of the culture since the number of bacterial cells can vary greatly between cultures. Too few cells (low cell mass) will result in low DNA yields and may cause a co-filtration of fine precipitates with cleared lysates when clearing of lysate using Ez-Clear™ filter. Conversely, with too many cells (high cell mass) the bacteria may not lyse efficiently and it causes poor release of the plasmid DNA resulting in a lower yield.

Use of rich broth such as Terrific Broth (TB) or 2xYT will lead to very high cell density. If these media were used, starting sample volume should be reduced not to overload GeneAll® plasmid kit column and buffer system. Alternatively, the volume of buffers including P1, P2, P3, 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 P1 in the presence of RNase A. Exposure of bacterial suspensions to the strongly anionic detergent at high pH (buffer P2, SDS/NaOH) opens the cell wall, denatures chromosomal DNA and proteins, and releases plasmid DNA into the supernatant. Although buffer P2, 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 conditions 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/P3 which replaces sodium ions by potassium ions.

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 alkaline lysis, the cellular debris and precipitates should be removed completely not to clog GeneAll® plasmid 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. EzClear™ Filter column can be assembled with GeneAll® plasmid binding column, and this column stack makes it one-step the clearance of lysate and the binding of plasmid DNA to SV column membrane when using vacuum as driving force. EzClear™ Filter Column is included only in GeneAll® Plasmid Midi series.

Removal of endotoxins

Endotoxins (known as lipopolysaccharides, LPS) can be efficiently removed during plasmid preparations by two steps; advanced phase separation and endotoxin-removal washing. After the addition of buffer ER to the lysate, the mixture is incubated on ice and then at 37°C, resulting in phase-separation of clear upper phase and blue bottom phase. While endotoxins transfer to the lower phase, plasmid DNA locates the upper phase. Because the phase can be dispersed after phase-separating, the mixture should be handled gently during transfer of the upper phase by pipetting. Repetition of this procedure will reduce not only the contaminaiton of endotoxins, but also the yield of plasmid DNA. Generally, the level of endotoxin may be less than 0.1 EU/ug after phase-separating and endotoxin-washing.

Washing

When working with *endA*$^+$ strains, endonucleases can be efficiently removed by first washing with buffer EW1 to ensure that plasmid DNA is not degraded during storage or enzyme reactions. Because buffer EW1 not only removes endonucleases but also enhances the quality of plasmid DNA by removal of residual proteins, it is essential especially when working with low-copy plasmids which are generally used with larger culture volume. buffer EW2 removes the salt and other cellular components bound nonspecifically to column membrane.

Elution

Purified DNA can be eluted in low salt buffer or deionized water as need for downstream applications. Buffer EF contains 10mM TrisCl, pH8.5 and is endotoxin-free. When using water as eluent, make sure that it is endotoxin-free and that the pH value is within 7.0 and 8.5. Because plasmid in water is susceptible to hydrolysis and lacks a buffering agent, it is recommended to store at -20°C.

The elution volume can be adjusted as necessity, but it has to be over the minimum requirement to soak completely the column membrane. For higher concentration of DNA, decrease the volume of elution buffer. For higher overall yield, increase the volume of elution buffer and repeat the elution step once again. The concentration and yield as the elution volume is shown below.

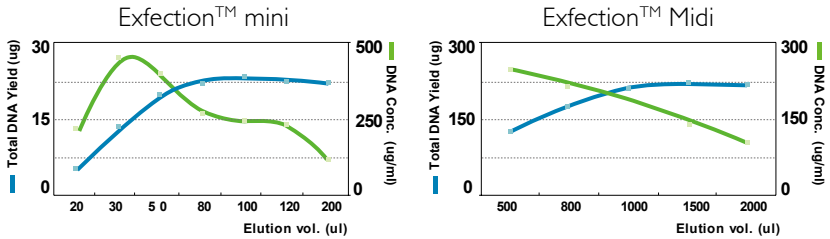


Fig. 2 The overall yield and concentration of plasmid DNA depending on the volume of elution. pUC18 plasmid DNA was purified from 3 ml (mini) and 40 ml (Midi) of overnight cultured DH5α using GeneAll® Exfection™ LE protocol. Plasmid DNA was eluted with the indicated volume of buffer EF.

Centrifuge for Midi Kits

GeneAll® Exfection™ Midi procedures require the conventional centrifuge which has a swinging-bucket rotor and ability of 4,000 ~ 5,000 xg. Use of fixed-angle rotor will cause inconsistent contact of column membrane with sample mixtures and buffers, and lead to unsatisfactory yield. Lower g-force may lead to not only uncomplete removal of ethanol, but also fail of eluting DNA from the membrane of spin column. Available centrifuges and rotors were listed below, but you can employ any equivalent.

Company	Centrifuge	Rotor
Beckman CoulterInc. (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

01 Exfection™ LE mini

Before Experiment

Prepare

Add RNase A to buffer P1 and store it 4°C.

Prepare 1.5 ml microcentrifuge tubes.

Temperature

All experiment should be performed at room temperature.

Centrifugation

Unless otherwise noted, all centrifugation steps should be performed at full speed (>12,000 xg or 12,000 rpm) in a microcentrifuge at room temperature.

Check

Buffer P2 and G3 may precipitate at cold ambient conditions. If precipitate appears, dissolve it in 37°C water bath.

Preparation of Cleared Lysate

1. **Pellet 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; up to 5 ml for high copy number plasmid, or up to 10 ml for low copy number plasmid. Bacterial culture should be grown for 16 to 24 hours in LB media containing selective antibiotics. 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 SV 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 the pelleted bacterial cells thoroughly in 250 ul of Buffer P1. Transfer the suspension to a new 1.5 ml tube.**
   It is essential to thoroughly resuspend the cell pellet.
   * Add RNase A to buffer P1 before first use.

3. **Add 250 ul of Buffer P2 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 P2 before use, heat to dissolve at 37°C (or above). Precipitated buffer P2 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.**

Isolation and Purification of plasmid DNA

6. **Transfer carefully the supernatant to a SV column by decanting or pipetting. Centrifuge for 30 sec. Remove the SV column, discard the pass-through, and re-insert the SV column to the collection tube.**
   Avoid the white precipitate co-transfering into the SV column.

7. **Apply 600 ul of Buffer EW1 and centrifuge for 30 sec. Remove the SV column, discard the pass-through, and re-insert the SV column to the collection tube.**
   This step will remove any traces of lipopolyssacharides (endotoxin), endonucleases, proteins, carbohydrates and other cellular components bound nonspecifically to the column membrane.

8. **Apply 700 ul of Buffer EW2 and centrifuge for 30 sec. Remove the SV column, discard the pass-through, and re-insert the SV column to the collection tube.**

9. **Centrifuge for an additional 1 min to remove residual wash buffer. Transfer the SV column to a new 1.5 ml tube (Not provided).**
   This step removes residual ethanol from SV column membrane. Residual ethanol in eluate may inhibit subsequent enzymatic reaction. If carryover of buffer EW2 occurs, centrifuge again for 1 min before proceeding to next step.

10. **Add 50 ul of Buffer EF or endotoxin-free water, let stand for 1 min, and centrifuge for 1 min.**
    Ensure that the buffer EF or endotoxin-free water is dispensed directly onto the center of SV column membrane for optimal elution of DNA. For the preparation from larger sample volume, elution volume can be increased to 200 ul maximum. It will increase the total yields of plasmid but decrease the concentration of eluate.
    For long-term storage, eluting in buffer EF (10mM TrisCl, pH 8.5) and storing at -20°C is recommended.

02 Exfection™ LE Midi

Before Experiment

Prepare

Add RNase A to buffer P1 and store it 4°C.

Temperature

All experiment should be performed at room temperature.

Centrifugation

Unless otherwise noted, all centrifugation steps should be performed at room temperature in a conventional centrifuge capable of 4,000 ~ 5,000 xg, which has a swinging-bucket rotor.

Check

Buffer P2 and G3 may precipitate at cold ambient conditions. If precipitate appears, dissolve it in 37°C water bath.

Preparation of Cleared Lysate

1. Pellet the 50 ml of 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; Up to 100 ml a bacterial culture can be used when $A_{600} < 1.8$. For the small sample less than 50 ml with an $A_{600} < 1.8$, decrease the volume of buffer P1, P2 and G3 to 2, 2 and 2.8 ml, respectively. If the cell-mass of starting sample is very low, some precipitates can be co-filtered through EzClear™ filter when clearing of lysate.

Bacterial culture should be grown for 16 to 24 hours in LB-broth containing a selective antibiotics. If other rich broth, such as TB or 2xYT, and/or higher culture volume than 100 ml is used, increase the volume of Buffer P1, P2 and G3 proportionally, since too high cell density of bacterial cells can cause the reduction of lysis efficiency, resulting in unsatisfactory yields.

2. Resuspend the pelleted bacterial cells thoroughly in 2.5 ml of Buffer P1.

It is essential to thoroughly resuspend the cell pellet.

* Add RNase A before first use of the Buffer P1.

3. Add 2.5 ml of Buffer P2 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 P2, heat to dissolve at 37°C (or above). Precipitated buffer P2 may cause significant decrease in DNA recover yield.

4. Add 3.5 ml of Buffer G3 and thoroughly but gently mix by inverting the tube 5-8 times (DO NOT VORTEX).

For better precipitation and adjustment of binding condition, mix the solution gently but completely and immediately after the addition of buffer G3.

Incubating on ice may help precipitate the denatured cell components more efficiently; and it may reduce the possibility of the contamination of chromosomal DNA.

Isolation and Purification of plasmid DNA

In this procedure, one of the two methods can be chosen to purify plasmid DNA. Plasmid DNA can be purified using centrifugation to pull the cleared lysate through the SV column. Alternatively, vacuum can be used to force the cleared lysate through the SV column.

A. Centrifugation Protocol

Always close the cap of the tube at centrifugation.

5. Pour all of the lysate or the cleared lysate into EzClear™ filter unit (blue ring) sitting on a 50 ml conical collection tube (provided). Incubate for 2 min and centrifuge for 2 min at 1,500 xg (2,800 rpm).

Cellular debris will rise to the top during incubation, and this will assist the clearing of lysate through EzClear™ Filter Unit. Failure to perform the incubation may lead to incomplete filtration of lysate. A small amount of liquid can remain trapped in the residual insoluble material, but this will not lead to noteworthy decrease in yield.

>> Optional centrifugation before this step

- If the cell mass of the bacterial culture is very dense (A_{600}>2.0) and the starting volume is larger than 50 ml, it may be necessary to centrifuge the alkaline lysate before transferring to EzClear™ filter. Because too high cell mass can cause the clogging of EzClear™ filter.
- This optional centrifugation can be done at 4,500 xg for 20 min on a swing-bucket rotor or at 10,000 xg for 10 min on a fixed angle rotor.
- After this optional centrifugation, transfer only the supernatant into EzClear™ filter. Some debris can be co-transfered.

6. Decant carefully the pass-through fraction to SV Midi column (clear ring). Centrifuge for 2 min at 1,500 xg (2,800 rpm). Remove the SV column, discard the pass-through, and re-insert the SV column to the collection tube.

7. Apply 10 ml of Buffer EW1 and centrifuge for 2 min at 1,500 xg (2,800 rpm). Remove the SV column, discard the pass-through, and re-insert the SV column to the collection tube.

This step will remove any traces of lipopolyssacharides (endotoxin), endonucleases, proteins, carbohydrates, and other cellular components bound non-specifically to the column membrane.

8. Apply 10 ml of Buffer EW2 and centrifuge for 2 min at 1,500 xg (2,800 rpm). Remove the SV column, discard the pass-through, and re-insert the SV column to the collection tube.

9. Apply 3 ml of Buffer EW2 and centrifuge for 15 min at 4,500 xg (5,000 rpm). Transfer the SV column to a new 50 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 from buffer EW2.

Residual ethanol in eluate may interfere with the subsequent reactions. If carryover of ethanol occurs, incubate the SV column for 10~20 min at room temperature to evaporate residual ethanol.

10. Add 0.6 ml of Buffer EF directly onto the center of the SV column membrane. Incubate for 5 min at room temperature and centrifuge for 5 min at 4,500 xg (5,000 rpm).

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

The volume of eluent can be increased or decreased as necessity. Higher volume will decrease the concentration of the eluate but yield slightly more DNA. The volume can be decreased but it should be 0.5 ml at least, because less volume is insufficient to soak the entire membrane.

For long-term storage, eluting in buffer EF (10mM TrisCl, pH 8.5) and storing at -20°C is recommended. Ensure that the pH of water is within 7.0 ~ 8.5, when using water for elution.

11. ***(optional :)***

A. For higher concentration of eluate; re-load the eluate from step 6 into the SV column membrane, close the cap, incubate 5 min at room temperature, and centrifuge for 5 min at 4,500 xg (5,000 rpm).

B. For more overall yield; add 0.6 ~ 1 ml of fresh buffer EF into the SV column, close the cap, incubate 5 min at room temperature, and centrifuge for 5 min at 4,500 xg (5,000 rpm).
The first and second eluates can be combined or collected separately as necessity.

B. Vacuum protocol

The vacuum pressure should be in this range; 23 ~ 26 inHg, 580 ~ 660 mmHg, 770 ~ 880 mbar or 11 ~ 12.5 psi. Lower vacuum pressure may reduce DNA yield and purity, and too high vacuum pressure may cause to burst the column membrane.

5. **Assemble a column stack by nesting EzClear™ Midi Filter Unit (blue-ring) into the top of SV Midi column (clear-ring). Attach the assembled column stack onto a port of the vacuum manifold tightly.**
   Most commercial vacuum manifold with luer connectors can be adopted to this protocol.

6. **Decant all of the lysate to EzClear™ Midi Filter Unit and incubate 1 ~ 3 min to allow the cellular debris and precipitates to rise to the top.**

7. **Apply maximum vacuum to draw the solution through the column stack. When all liquid has been pulled through the SV Midi column at the bottom, slowly release the vacuum.**
   The lysate will pass through EzClear™ Filter Unit and plasmid DNA will bind the membrane in Midi SV column.
   If some of the lysate does not pass through the EzClear™ Filter Unit, remove the filter unit, place it into a new 50 ml conical tube, and centrifuge for 3 min at 1,750 xg (3,000 rpm). Then apply the pass-through to the Midi SV column.
   If the vacuum is released too quickly, the membrane may detach from the SV column. If the membrane becomes detached, tap it down gently with something sterile.

8. **Discard the upper EzClear™ Filter Unit (blue) and apply 10 ml of Buffer EW1 to SV Midi column (clear). Switch on vacuum source to draw the solution through the SV Midi column and slowly release the vacuum.**
   This step will remove any traces of endotoxin, endonucleses, proteins, carbohydrates, and other cellular components bound nonspecifically to the SV column membrane.

9. **Apply 14 ml of Buffer EW2 and switch on vacuum source. When all liquid has been pulled through the SV Midi Column, slowly release the vacuum.**

10. **Transfer the SV Midi Column to a 50 ml conical tube (provided).**

11. **Go to step 9 in Centrifugation Protocol (page 24).**

03 Exfection™ EF Midi

Before Experiment

Prepare

Add RNase A to buffer P1 and store it 4°C.

Chill buffer P3 for better result. Buffer P3 can be stored at 2 ~ 8°C without any precipitation.

Temperature

Prepare Ice.

Prepare 37°C water bath or incubator.

All experiment should be performed at room temperature.

Centrifugation

Unless otherwise noted, all centrifugation steps should be performed at room temperature in a conventional centrifuge capable of 4,000 ~ 5,000 xg, which has a swinging-bucket rotor.

Check

Buffer P2 and EG may precipitate at cold ambient conditions. If precipitate appears, dissolve it in 37°C water bath.

Preparation of Cleared Lysate

1. Pellet 50 ~ 100 ml of 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; Up to 150 ml of a bacterial culture can be used when the A_{600} is less than 1.8. For the bacterial culture with high cell density (A_{600}>2.0), reduce the starting sample volume to 50 ml. Too high cell mass of starting sample can cause the reduction of lysis efficiency and the clogging of the columns, resulting in unsatisfactory yields.

Bacterial culture should be grown for 16 to 24 hours in LB-broth containing selective antibiotics.

2. Resuspend pelleted bacterial cells thoroughly in 4 ml of Buffer P1.

It is essential to thoroughly resuspend the cell pellet.

* Add RNase A before first use of buffer P1.

3. Add 4 ml of Buffer P2 and mix by inverting the tube 5 ~ 6 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 the bottle of buffer P2, heat to dissolve at 37°C (or above). Use of precipitated buffer P2 may cause significant decrease in DNA recover yield.

Keep buffer P2 capped tightly after use because it can be acidified gradually when in contact with air.

4. Add 4 ml of Buffer P3 and thoroughly but gently mix by inverting the tube 7 ~ 8 times (DO NOT VORTEX).

Mix the solution gently but completely and immediately after addition of buffer P3 for optimal precipitation.

Use of prechilled P3 or Incubation on ice may help precipitate the denatured cell components more efficiently; and it may reduce the possibility of the contamination of chromosomal DNA.

5. Pour all of the lysate into EzClear™ filter (blue ring) sitting on a 50 ml conical collection tube (provided). Let it stand for 2 min and centrifuge for 2 min at 1,500 xg (2,800 rpm).

Cellular debris will rise to the top during incubation, and this will assist the clearing of lysate through EzClear™ Filter. Failure to perform the incubation may lead to incomplete filtration of lysate. A small amount of liquid can remain trapped in the residual insoluble material, but this will not lead to noteworthy decrease in yield.

>> Optional centrifugation before this step

- If the cell mass of the bacterial culture is very dense (A_{600}>2.0) and the starting volume is larger than 100 ml, it may be necessary to centrifuge the lysate before transferring to EzClear™ filter. Because too high cell mass can cause the clogging of EzClear™ filter on next step.
- This optional centrifugation can be done at 4,500 xg for 20 min on a swing-bucket rotor or at 10,000 xg for 10 min on a fixed-angle rotor.
- After this optional centrifugation, transfer the supernatant into EzClear™ Filter Unit (Some debris can be co-transfered).

Removal of endotoxin

6. Apply 500 ul of buffer ER to the filtrate and close the cap of 50 ml conical tube.

The volume of filtrate may be about 10 ml.

7. Vortex to mix and incubate for 15 min on ice.

The mixture will be turbid with vortexing and then become clear during incubation on ice.

8. Incubate for 15 min at 37˚C and centrifuge for 2 min at 1,500 xg (2,800 rpm).

After centrifugation, the mixture will be separated by two phases: the white upper phase and the blue (greenish) lower phase. Handle the tube gently not to break up the phase.

9. Transfer carefully the upper phase (clear) into a fresh 15 ml conical tube (provided) by pipetting.

Be careful not to co-transfer the lower phase (greenish blue) since it is liable to be dispersed.

If phase is broken up, repeat centrifugation again.

Isolation and Purification of plasmid DNA

Always close the cap of the tube at centrifugation.

10. Add 1/2 vol of Buffer EG to the transfer and invert several times to mix completely.

For 10 ml of the solution, 5 ml of buffer EG should be added.

11. Transfer all of the mixture to SV Midi column (clear ring) by decanting or pipetting. Centrifuge for 2 min at 1,500 xg (2,800 rpm). Remove the Column, discard the pass-through, and re-insert the column to the collection tube.

12. Apply 10 ml of Buffer EW1 and centrifuge for 2 min at 1,500 xg (2,800 rpm). Remove the Column, discard the pass-through, and re-insert the Column to the collection tube.

This step will remove any traces of lipopolyssacharides (endotoxin), endonucleses, proteins, carbohydrates, and other cellular components bound nonspecifically to the column membrane.

13. Apply 10 ml of Buffer EW2 and centrifuge for 2 min at 1,500 xg (2,800 rpm). Remove the Column, discard the pass-through, and re-insert the Column to the collection tube.

14. Apply 3 ml of Buffer EW2 and centrifuge for 15 min at 4,500 xg (5,000 rpm). Transfer the Column to a new 50 ml conical tube (provided).

Care must be taken at the removal of SV Midi 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 from buffer EW2.

Residual ethanol in eluate may interfere with the subsequent reactions. If carryover of ethanol occurs, incubate the column for 10 ~ 20 min at room temperature to evaporate residual ethanol.

15. Add 0.6 ml of Buffer EF or endotoxin-free water directly onto the center of the Column membrane and close the cap. Incubate for 5 min at room temperature and centrifuge for 5 min at 4,500 xg (5,000 rpm).

Ensure that the elution buffer is dispensed directly onto the center of column membrane for optimal elution of DNA.

The volume of eluent can be increased or decreased as necessity. Higher volume will decrease the concentration of the eluate but yield slightly more DNA. The volume can be decreased but it should be 0.5 ml at least, because less volume is insufficient to soak the entire membrane.

For long-term storage of DNA, other buffers, such as TE (1 mM EDTA, 10 mM TrisCl, pH 8.0), can be used for elution. The buffer for elution should be neutral pH (7.0<pH<9.0) and low-salt condition.

Ensure that the solution and the plastic-ware for elution is endotoxin-free.

16. *(optional :)*

A. **For higher concentration of eluate; re-load the eluate from step 15 into the column membrane, close the cap, incubate 5 min at room temperature, and centrifuge for 5 min at 4,500 xg (5,000 rpm).**

B. **For more overall yield; add 0.6~1 ml of fresh buffer EF into the column, close the cap, incubate 5 min at room temperature, and centrifuge for 5 min at 4,500 xg (5,000 rpm).**

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

Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low or no yield of plasmid DNA	**Too many cells in sample**	Cultures should be grown for 16 ~ 24 hours in proper media with antibiotics. Reduce the culture volume of sample. If rich broth such as Terrific Broth (TB) or 2xYT is used, starting sample volume must be reduced because these media have very high cell density (2 ~ 5 times to LB).
	Too few cells in sample	Confirm cell density by taking absorbance at 600 nm. Use more cells.
	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.
	Too old sample	Streak a fresh plate from a freezer stock. Pick a single colony and prepare a new culture.
	Insufficient antibiotic activity	Confirm that the appropriate amount of fresh antibiotic was present during growth of culture. Most antibiotics are light sensitive and degrade during long-term storage at 2 ~ 8°C. Use fresh antibiotics.
	Poor resuspension of bacterial pellets in Buffer P1	Bacterial cell pellets must be thoroughly resuspended in buffer P1
	Buffer P2 precipitated	Redissolve buffer P2 by warming to 37°C
	Insufficient digestion with RNase	Excessive RNA can interfere the binding of plasmid DNA with GeneAll® spin column membrane. If buffer P1 containing RNase is more than a year old, the activity of RNase can be decreased. Add additional RNase in such a case. (Working concentration = 100 ug/ml)

Facts	Possible Causes	Suggestions
	Inadequate elution buffer	DNA can be eluted only by low salt condition. Buffer EF (10mM TrisCl, pH8.5) has the optimal elution efficiency, but other eluent 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.
	Improper centrifuge (Midi)	Swing-bucket rotor (capable of 4,000 ~ 5,000 xg) must be used. Use of fixed-angle rotor may lead to failure of proper contact between cleared lysate and a column membrane, resulting in poor and inconsistent yield of DNA.
Low purity	**Contamination of precipitate when binding**	When the cleared lysate is transferred to GeneAll® plasmid kit column, ensure that any precipitate does not contain to the transfer.
	Improper centrifuge (Midi)	Swing-bucket rotor (capable of 4,000 ~ 5,000 xg) must be used. Do not use fixed angle rotor.
Lysate is not clear after filtration (LE Midi)	**Precipitation may have occurred**	When working with culture volumes less than 50 ml, or with low cell-density cultures (A_{600} < 1.8), excessive SDS may be precipitated by substitution of sodium with potassium ions. This fine precipitate can be co-filtered with cleared lysate. When using cultures containing low-cell mass, decrease the buffers for lysis (P1, P2, G3) as described in the annotation of step 1 at page 20.
The phase is reversed after phase-separating or the color of upper phase is greenish-blue (EF Midi)	**The biomass used is too much.**	Reduce the starting sample. The high density of cleared lysate will reverse the phase.

Facts	Possible Causes	Suggestions
Chromosomal DNA contamination	**Mis-handling of the lysate after addition of buffer G3/P3**	Vigorous vortexing after the addition of buffer G3/P3 can cause shearing of DNA followed by chromosomal DNA contamination. Handle gently the lysate after the addition of buffer G3/P3. Inverting and rotating tube to cover walls with lysate is sufficient for mixing. But it should be thorough to neutralize the whole lysate.
Smearing of plasmid DNA; additional band behind or ahead of plasmid DNA	**Too long lysis time**	Too long lysis time in buffer P2 can cause denaturation of supercoiled plasmid DNA. Proceed to next step immediately after no more clumps are visible in the lysate. Lysis time should not be over 5 minutes in any case. A small amount of this species of DNA is common and is suitable for downstream applications.
	Vigorous mixing in buffer P2	Vigorous handling after the addition of buffer P2 can lead to irreversible denaturation of plasmid DNA. Gentle inverting and rotating tube to cover walls with viscous lysate is sufficient for mixing.
RNA Contamination	**RNase omitted or old**	RNase solution should be added to buffer P1 before first use. If buffer P1 containing RNase is more than a year old, the activity of RNase can be decreased. Add additional RNase (working concentration = 100 ug/ml). Buffer P1 containing RNase should be stored at 4°C.
	Too many cells in sample	Reduce the sample volume. Too many cells may not be subjected properly to RNase digestion.
High salt concentration in eluate	**Improper wash step**	Ensure that washing steps are performed properly. The column membrane should be completely dried via additional centrifugation or air-drying for good result.

Facts	Possible Causes	Suggestions
DNA floats out of well while loading of agarose gel	**Alcohol included in wash buffer is not completely removed during wash steps**	Ensure the wash step in protocols. Supplementarily, incubate for 5 minutes at room temperature after applying buffer EW2 in wash steps.
Enzymatic reaction is not performed well with purified DNA	**High salt concentration in eluate**	Ensure that washing steps have been carried out just in accordance with the protocols. Repeat of washing steps may help to remove high salt in eluate. Supplementarily, incubate for 5 minutes at room temperature after applying buffer EW2 in wash steps.
	Prepared plasmid DNA is permanently denatured	Do not allow the lysis reaction in buffer P2 to proceed longer than 5 minutes. Prolonged alkaline lysis may permanently denature plasmid DNA.
	Residual alcohol in eluate	Ensure that the washing steps have been performed properly. The 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	50 200	mini / spin	100-150 100-102
GeneAll® *Exprep™* *for preparation of plasmid DNA*			
Plasmid SV mini	50 200 1,000	spin / vacuum	101-150 101-102 101-111
Plasmid SV Midi**	26 50 100	spin / vacuum	101-226 101-250 101-201
Plasmid SV Quick	50 200 1,000	mini / spin	101-050 101-002 101-011
GeneAll® *Exfection™* *for preparation of highly pure plasmid DNA*			
Plasmid LE mini *(Low Endotoxin)*	50 200	spin / vacuum	111-150 111-102
Plasmid LE Midi* *(Low Endotoxin)*	26 100	spin / vacuum	111-226 111-201
Plasmid EF Midi* *(Endotoxin Free)*	20 100	spin	121-220 121-201
GeneAll® *Expin™* *for purification of fragment DNA*			
Gel SV	50 200	mini / spin / vacuum	102-150 102-102
PCR SV	50 200	mini / spin / vacuum	103-150 103-102
CleanUp SV	50 200	mini / spin / vacuum	113-150 113-102
Combo GP	50 200	mini / spin / vacuum	112-150 112-102
GeneAll® *Exgene™* *for isolation of total DNA*			
Tissue SV mini (*plus!*)*	100 250	spin / vacuum	104(9)-101 104(9)-152
Tissue SV Midi (*plus!*)**	26 100	spin / vacuum	104(9)-226 104(9)-201
Tissue SV MAXI (*plus!*)**	10 26	spin / vacuum	104(9)-310 104(9)-326
Blood SV mini	100 250	spin / vacuum	105-101 105-152
Blood SV Midi**	26 100	spin / vacuum	105-226 105-201
Blood SV MAXI**	10 26	spin / vacuum	105-310 105-326

Ordering Information

Products	Size	Type	Cat. No.
GeneAll® *Exgene™* *for isolation of total DNA*			
Cell SV mini	100 250	spin / vacuum	106-101 106-152
Cell SV MAXI**	10 26	spin / vacuum	106-310 106-326
Clinic SV mini	100 250	spin / vacuum	108-101 108-152
Clinic SV Midi	26 100	spin / vacuum	108-226 108-201
Clinic SV MAXI**	10 26	spin / vacuum	108-310 108-326
Plant SV mini	100 250	spin / vacuum	117-101 117-152
Plant SV Midi**	26 100	spin / vacuum	117-226 117-201
Plant SV MAXI**	10 26	spin / vacuum	117-310 117-326
GMO SV mini	50 200	spin / vacuum	107-150 107-102
GeneAll® *GenEx™* *for isolation of total DNA*			
GenEx™ B	100† 500† 100††	mini / solution mini / solution MAXI / solution	220-101 220-105 220-301
GenEx™ C	100† 500† 100††	mini / solution mini / solution MAXI / solution	221-101 221-105 221-301
GenEx™ T	100† 500† 100††	mini / solution mini / solution MAXI / solution	222-101 222-105 222-301
GeneAll® *RiboEx™* *for preparation of total RNA*			
RiboEx™	100 200	solution	301-001 301-002
Hybrid-R™	100	spin	305-150
RiboEx™ LS	100 200	solution	302-001 302-002
Riboclear™	50	spin	303-150
Ribospin ™	50	spin	304-150
Ribospin vRD™	50	spin	302-150
Allspin™	50	spin	306-150

Products	Size	Type	Cat. No.
GeneAll® *AmpONE™* *for PCR amplification*			
Taq DNA polymerase	250 U 500 U 1,000 U	(2.5 U/㎕)	501-025 501-050 501-100
α-Taq DNA polymerase	250 U 500 U 1,000 U	(2.5 U/㎕)	502-025 502-050 502-100
Pfu DNA polymerase	250 U 500 U 1,000 U	(2.5 U/㎕)	503-025 503-050 503-100
Hotstart Taq DNA polymerase	250 U 500 U 1,000 U	(2.5 U/㎕)	531-025 531-050 531-100
Clean Taq DNA polymerase	250 U 500 U 1,000 U	(2.5 U/㎕)	551-025 551-050 551-100
Clean α-Taq DNA polymerase	250 U 500 U 1,000 U	(2.5 U/㎕)	552-025 552-050 552-100
Taq Master mix	2x 2x	0.5 ml x 2 tubes 0.5 ml x 10 tubes	511-010 511-050
α-Taq Master mix	2x 2x	0.5 ml x 2 tubes 0.5 ml x 10 tubes	512-010 512-050
Taq Premix	20 ㎕ 50 ㎕	96 tubes	521-200 521-500
α-Taq Premix	20 ㎕ 50 ㎕	96 tubes	522-200 522-500
Taq Premix (w/o dye)	20 ㎕	96 tubes	524-200
α-Taq Premix (w/o dye)	20 ㎕	96 tubes	525-200
dNTP mix	500 ㎕	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, 2 x 10^6 cells or 10 mg animal tissue.
†† On the basis of DNA purification from 10 ml whole blood. 1 x 10^8 cells or 100 mg animal tissue.

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

Edited by BnP
Designed by Joo Sang Mi