Ver 7.0

Handbook for

PLASMID SV MINI
PLASMID SV MIDI

exprep™

DNA PURIFICATION HANDBOOK

GeneAll

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This protocol handbook is included in :

GeneAll® Exprep™ Plasmid SV mini (101-150, 101-102)
GeneAll® Exprep™ Plasmid SV Midi (101-226, 101-250, 101-201)

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

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	Exprep™ Plasmid SV mini	
Cat. No.	**101-150**	**101-102**
Size	**mini**	**mini**
No. of preparation	50	200
Column Type Q (with collection tube)	50	200
Buffer S1	20 ml	60 ml
Buffer S2	20 ml	60 ml
Buffer S3	25 ml	90 ml
Buffer AW (concentrate) *	19 ml	69 ml
Buffer PW (concentrate) * †	12 ml	50 ml
Buffer EB **	15 ml	30 ml
RNase A (20 mg/ml)	100 µl	300 µl
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	Exprep™ Plasmid SV Midi		
Cat. No.	**101-226**	**101-250**	**101-201**
Size	**Midi**	**Midi**	**Midi**
No. of preparation	26		
Column Type Q (red ring) (with collection tube)	26		
EzClear™ Filter (blue ring) (with collection tube)	26		
Buffer S1	80 ml		
Buffer S2	80 ml	**101-226 × 2**	**101-226 × 4**
Buffer S3	110 ml		
Buffer AW (concentrate) *	94 ml x 2		
Buffer PW (concentrate) * †	50 ml x 2		
Buffer EB **	120 ml		
RNase A (20 mg/ml)	400 µl		
Mix Vu™	110 µl		
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** Before using for the first time, add absolute ethanol (ACS grade or better) into Buffer AW and PW as indicated on the bottle.*

† Contains sodium azide as a preservative

*** 10 mM TrisCl, pH 8.5*

Quality Control

All components in Exprep™ Plasmid SV 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 approved.

Storage Conditions

Exprep™ Plasmid SV 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 S3 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.

Chemical Hazard

The buffers included in Exprep™ Plasmid SV kit contain the irritants which are harmful when directly exposed to skin, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector, and follow standard safety precautions.
Buffer S3 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.

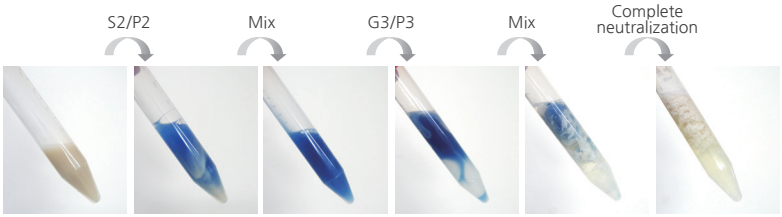
pH Indicator

Mix Vu™ is a visual pH indicator that monitors pH shifts during alkaline lysis, ensuring proper mixing to prevent errors. This helps achieve complete neutralization and consistent yield.

Mix Vu™ should be added at a 1:1000 ratio (e.g., 1 μl per 1 ml suspension). For Exprep™ Midi or Exfection™ LE Midi, use 2.5 μl per prep, and for Exfection™ EF Midi, use 4 μl per prep. Vortex thoroughly after adding Mix Vu™.

Alternatively, Mix Vu™ can be pre-mixed with S1(P1) Buffer at a 1:1000 ratio (e.g., 80 μl Mix Vu™ into 80 ml S1(P1) Buffer or 100 μl into 100 ml P1 Buffer). **Since Mix Vu™ does not fully dissolve in S1(P1) Buffer, shake the buffer before use to resuspend any precipitated particles.**

The solution turns blue upon adding S2(P2) Buffer, indicating complete alkalization. Mix thoroughly until the color is uniform. After adding G3(P3) Buffer, the solution becomes colorless, confirming full neutralization. If any color remains, mix further before proceeding.



Product Specifications

	Exprep™ Plasmid SV	
	mini	**Midi***
Format	Spin/Vacuum	Spin/Vacuum
Recommended sample volume	2~10 ml	50 ml
Maximum sample volume	10 ml	100 ml
Clearing of lysate	Centrifuge	EzClear™
Preparation time	<23 min	<50 min
Maximum loading volume	800 μl	15 ml
Binding capacity	30 μg	300 μg
Recovery rate	85~95%	85~95%
Minimum elution volume	40 μl	400 μl

* Exprep™ Plasmid SV Midi kit procedure requires the centrifuge which has a swinging-out bucket and ability of 4,000~5,000 x g.

EXPREP™ PLASMID DNA PURIFICATION KIT

Introduction

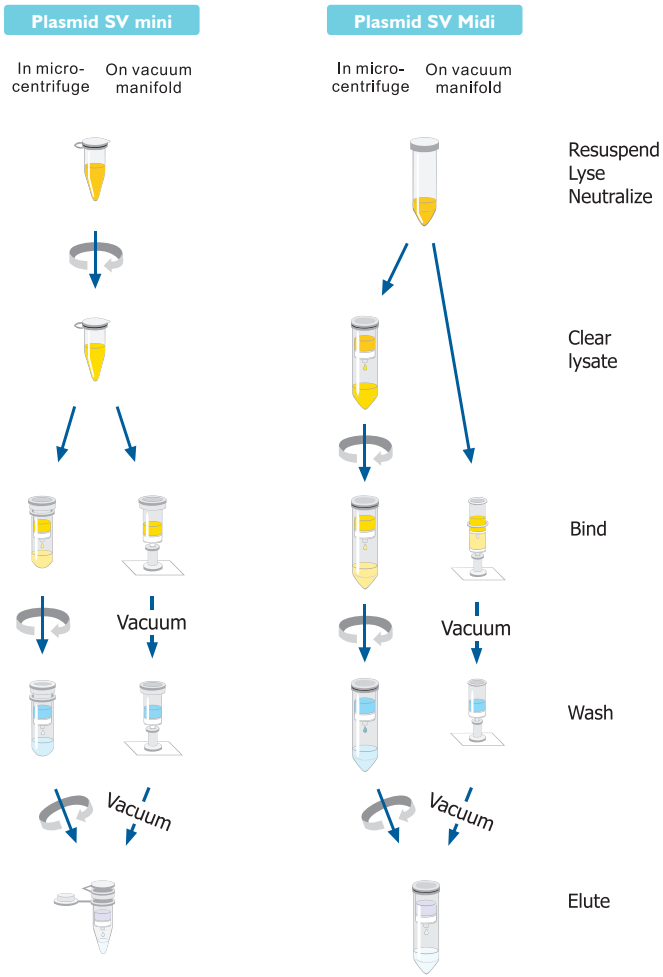
Exprep™ Plasmid SV kit provides easy and rapid method for the small and the medium scale preparation of plasmid DNA from bacterial cells. This kit can be used to isolate and purify any plasmid, but works most efficiently when the plasmid is less than 20 kb in size.

All process to prepare pure plasmid DNA takes only about 25 min and simultaneous processing of multiple samples can be easily performed. Up to 30 μg of pure plasmid can be purified using Exprep™ Plasmid SV mini 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

Exprep™ Plasmid SV 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 centrifugation for mini kit and by EzClear™ Filter for Midi kit.

In the presence of high salt, plasmid DNA in cleared lysate binds selectively to glass microfiber membrane in Column Type Q. 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.



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. 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, 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.

Exprep™ Plasmid SV kit procedure is optimized to high-copy number plasmid, so larger starting sample may be needed if low-copy number plasmids are used.

Table1. 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 DH5α and XL1-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 13). If endonuclease I is not completely removed during plasmid preparations, the plasmid DNA in eluate is degraded during subsequent incubation in the presence of Mg^{2+} (e.g. during incubation with restriction enzyme). This problem can be avoided by use of *endA*$^-$ strains (denoted as *endA1*) such as DH5α and XL1-Blue. Extra wash with Buffer AW will also help prevent the degradation of DNA.

Exprep™ series 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 Exprep™ Plasmid SV column and buffer system. Otherwise, the volume of Buffer S1, S2 and S3 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 S3 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

After mixing with Buffer S3 the cellular debris and precipitates should be removed completely not to clog Column Type Q in subsequent binding. New patented EzClear™ Filter facilitates the clearance of the lysate by filtration instead of tedious centrifugation which has been used widely in traditional methods.

EzClear™ Filter is included in Exprep™ Plasmid SV Midi kit.

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 necessary when working with low-copy plasmids which are generally used with larger culture volume. Buffer PW removes the salt and other cellular components bound nonspecifically to Column Type Q membrane.

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

***EndA*$^+$ strains**	***EndA*$^-$ strains**
BL21(DE3), CJ236, HB101, JM83, JM101, JM110, LE392, MC1061, NM series, P2392 PR series, RR1, TB1, TG1, BMH71-18, ES1301, wild-type and etc.	DH1, DH20, DH21, DH5α, JM103, JM105, JM106, JM107, JM108, JM109, MM294, SK1590, SRB, XL1-Blue, XLO and etc.

Elution

Purified DNA can be eluted in low salt buffer or deionized water as need for downstream applications. Buffer EB contains 10 mM TrisCl, pH 8.5. When using water as eluent, make sure 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 below -20°C. The elution volume can be adjusted as necessity, but it has to be over the minimum requirement to soak completely the Column Type Q membrane. For higher concentration of DNA, decrease the volume of elution buffer. For higher 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.

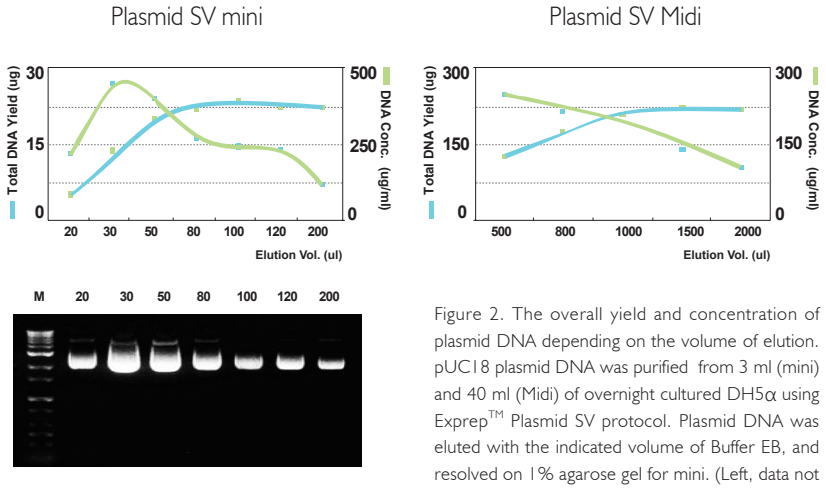


Figure 2. The overall yield and concentration of plasmid DNA depending on the volume of elution. pUC18 plasmid DNA was purified from 3 ml (mini) and 40 ml (Midi) of overnight cultured DH5α using Exprep™ Plasmid SV protocol. Plasmid DNA was eluted with the indicated volume of Buffer EB, and resolved on 1% agarose gel for mini. (Left, data not shown for Midi)

Centrifuge in Midi kit protocol

Exprep™ Plasmid SV Midi procedures require the conventional centrifuge which has a swinging-bucket rotor and ability of 4,000~5,000 x g.

Use of fixed-angle rotor will cause inconsistent contact of Column Type Q (Midi) membrane with sample mixtures and buffers, and lead to unsatisfactory result.

Low g-force may lead to not only uncomplete removal of ethanol, but also fail of eluting DNA from the membrane of 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

Exprep™ Plasmid SV mini

Before Experiment

* *Before using for the first time, add absolute ethanol (ACS grade or better) into Buffer AW and PW as indicated on the bottle.*
* *Unless there is an another indication, all centrifugation steps should be performed at full speed (>10,000 x g or 10,000~14,000 rpm) in a microcentrifuge at room temperature.*
* *Add all of RNase A to Buffer S1 before first use.*
* *Store the Buffer S1 at 4°C after addition of RNase A.*
* *Prepare new 1.5 ml or 2 ml microcentrifuge tubes.*
* *Buffer S2 and S3 may precipitate at cool ambient conditions. If precipitate appears, dissolve it in 37°C water bath until completely dissolved.*

Preparation of Cleared Lysate

1. Pellet the bacterial culture by centrifugation for 5 min at 10,000 x g in a centrifuge. Discard the supernatant as much as possible.

Use 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 21 hours in LB media containing a 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 Column Type Q (mini), 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 μl of Buffer S1. Transfer the suspension to a new 1.5 ml microcentrifuge tube.

It is essential to thoroughly resuspend the cell pellet.

You don't need to transfer the suspension if the cells have been pelleted in an 1.5 ml microcentrifuge tube at previous step.

* Add RNase A to Buffer S1 before first use.

3. Add 250 μl 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 μl of Buffer S3 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 S3.

5. Centrifuge for 10 min at full speed.

Isolation and Purification of Plasmid DNA

When using this kit, 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 Column Type Q (mini). Alternatively, vacuum can be used to force the cleared lysate through the column (page 20).

A Centrifugation Protocol

1. **Transfer carefully the supernatant to a Column Type Q (mini) by decanting or pipetting. Centrifuge for 30 sec at full speed. Remove the column, discard the pass-through, and re-insert the column to the collection tube.**
   Avoid the white precipitate co-transferring into the column.

2. ***(Optional:)* Apply 500 µl of Buffer AW and centrifuge for 30 sec at full speed. Remove the Column Type Q (mini), discard the pass-through, and re-insert the 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 *endA*1 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 *endA*1 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 13. When low-copy plasmid is used, it is strongly recommended to carry out this step, even though *endA*$^-$ strains.

3. **Apply 700 µl of Buffer PW and centrifuge for 30 sec at full speed. Remove the Column Type Q (mini), discard the pass-through, and re-insert the column to the collection tube.**

4. Centrifuge for an additional 1 min to remove residual wash buffer. Transfer the Column Type Q (mini) to a new 1.5 ml microcentrifuge tube (not provided).

This step removes residual ethanol from 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.

5. Add 50 μl 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 Column Type Q (mini) membrane for optimal elution of DNA.

Eluent volume can be adjusted to 200 μl maximum and it will increase the total yield of plasmid but decrease the concentration of eluate. For higher concentration of eluate, eluent volume can be decreased to 40 μl minimum.

The volume of eluate can be smaller than that of eluent and it will not effect the yield.

For long-term storage, eluting in Buffer EB (10 mM 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 ddH_2O is applied for elution. Incubate for 2 min after addition of pre-heated elution buffer.

B Vacuum Protocol

15~18 in Hg
285~345 mm Hg
380~460 mbar
5.5~6.5 psi

Pressure range
The vacuum pressure should be in the range of this list. Lower vacuum may reduce DNA yield and purity, and too high vacuum pressure may cause to burst the Column Type Q (mini) membrane.

1. **Attach the Column Type Q to a port of the vacuum manifold tightly.**
   Most commercial vacuum manifold with luer connectors can be used.

2. **Transfer the cleared lysate to the Column Type Q (mini), by pipetting or decanting.**
   Care should be taken not to transfer any of the white precipitate with the supernatant.

3. **Switch on vacuum source to draw the solution through the Column Type Q (mini). When all liquid has been pulled through the column, release the vacuum.**

4. ***(Optional:)* Apply 500 µl of Buffer AW. Switch on vacuum source to draw the solution through the Column Type Q (mini) and switch off the vacuum source.**
   See the annotation of step 2 in 'Centrifugation Protocol' at page 18.

5. **Apply 800 µl of Buffer PW and switch on vacuum source. When all liquid has been pulled through the Column Type Q (mini), release the vacuum.**

6. **Transfer the Column Type Q (mini) to a collection tube (provided).**

7. **Go to step 4 in 'Centrifugation Protocol' (page 19).**

Exprep™ Plasmid SV Midi

Before Experiment

* *Before using for the first time, add absolute ethanol (ACS grade or better) into Buffer AW and PW as indicated on the bottle.*
* *Unless there is an another indication, all centrifugation steps should be performed at room temperature in a centrifuge capable of 4,000~5,000 x g, which has a swinging-bucket rotor (See page 15).*
* *Add all of RNase A to Buffer S1 before first use.*
* *Store the Buffer S1 at 4°C after addition of RNase A.*
* *Buffer S2 and S3 may precipitate at cool ambient conditions. If precipitate appears, dissolve it in 37°C until completely dissolved.*

Preparation of Cleared Lysate

1. Pellet the 50 ml of bacterial culture by centrifugation for 5 min at 10,000 x g in a tabletop centrifuge. Discard the supernatant as much as possible.

Use appropriate volume of bacterial cultures; for the small sample less than 50 ml or the sample of 50 ml with an OD_{600}<2, decrease the volume of Buffer S1, S2 and S3 to 2, 2 and 2.8 ml, respectively.

Bacterial culture should be grown for 16 to 21 hours in LB-broth containing a selective antibiotics. If other rich broth, such as TB or 2xYT, and/or higher culture volume more than 100 ml is used, increase the volume of Buffer S1, S2 and S3 proportionally, since too high cell density of bacterial cells can cause the reduction of lysis efficiency, resulting in unsatisfactory yields.

2. Resuspend pelleted bacterial cells thoroughly in 2.5 ml of Buffer S1.

It is essential to thoroughly resuspend the cell pellet.

* Add RNase A before first use of the Buffer S1.

3. Add 2.5 ml 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, heat to dissolve at 37°C. Precipitated Buffer S2 may cause significant decrease in DNA recover yield.

4. Add 3.5 ml of Buffer S3 and thoroughly but gently mix by inverting the tube 4~6 times (DO NOT VORTEX).

For better precipitation and adjustment of binding condition, mix the solution gently but completely and immediately after addition of Buffer S3.

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. *(Optional:)* Centrifuge for 20 min at 4,500 x g (5,000 rpm).

Alternatively, centrifuge for 10 min at 10,000 x g (9,000 rpm) on fixed-angle-rotor centrifuge.

Because too high cell density of bacterial cells can cause the clogging of EzClear™ Filter (Midi, blue ring) on next step, this step may be necessary for large or dense sample.

Isolation and Purification of Plasmid DNA

When using this kit, 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 Column Type Q (Midi). Alternatively, vacuum can be used to force the cleared lysate through the column (page 26).

A Centrifugation Protocol

1. **Pour all of the lysate or the cleared lysate into EzClear™ Filter (Midi, blue ring) unit sitting on a 50 ml conical collection tube (provided). Incubate for 2 min and centrifuge for 3 min at 1,000 x g (2,200 rpm).**
   Cellular debris will rise to the top during incubation, and this will assist the clearing of lysate through 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.
   If the optional centrifugation is performed on step 5 at page 22, transfer only the supernatant into filter unit (Some debris can be co-transferred).

2. **Decant carefully the pass-through fraction to Column Type Q (Midi, red ring). Centrifuge for 3 min at 1,000 x g (2,200 rpm). Remove the column, discard the pass-through, and re-insert the column to the collection tube.**

3. **Apply 9 ml of Buffer AW and centrifuge for 3 min at 1,000 x g (2,200 rpm). Remove the Column Type Q (Midi, red ring), discard the pass-through, and re-insert the column to the collection tube.**
   This step will remove any traces of proteins, carbohydrates, and other cellular components bound nonspecifically to the column membrane.

4. **Apply 12 ml of Buffer PW and centrifuge for 3 min at 1,000 x g (2,200 rpm). Remove the Column Type Q (Midi, red ring), discard the pass-through, and re-insert the column to the collection tube.**

5. Apply 3 ml of Buffer PW and centrifuge for 15 min at 4,500 x g (5,000 rpm). Transfer the Column Type Q (Midi, red ring) to a new 50 ml conical tube (not provided).

Care must be taken at the removal of 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 PW.

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

6. Add 0.6 ml of Buffer EB or deionized distilled water directly onto the center of the Column Type Q (Midi, red ring) membrane. Incubate for 5 min at room temperature and centrifuge for 5 min at 4,500 x g (5,000 rpm).

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

The volume of eluent can be decreased to 400 μl for higher concentration of DNA, but this will slightly decrease in overall DNA yield. On the contrary, larger elution-volume will decrease the concentration of eluate but yield slightly more DNA.

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

Some larger plasmids (>10 kb) usually may not be eluted optimally unless pre-heated (70°C) Buffer EB or ddH_2O is applied for elution. Incubate for 2 min after addition of pre-heated elution buffer.

7. *(Optional:)*

A. For higher concentration of eluate; re-load the eluate from step 6 into the Column Type Q (Midi, red ring) membrane, close the cap, incubate 5 min at room temperature, and centrifuge for 5 min at 4,500 x g (5,000 rpm).

B. For more overall yield; add 0.6~1 ml of fresh Buffer EB into the Column Type Q (Midi, red ring), close the cap, incubate 5 min at room temperature, and centrifuge for 5 min at 4,500 x g (5,000 rpm).

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

B Vacuum Protocol

23~26 in Hg
580~660 mm Hg
77~880 mbar
11~12.5 psi

Pressure range
The vacuum pressure should be in the range of this list. Lower vacuum pressure may reduce DNA yield and purity, and too high vacuum pressure may cause to burst the Column Type Q (Midi, red ring) membrane.

1. **Assemble a column stack by nesting EzClear™ Filter (Midi, blue ring) unit into the top of Column Type Q (Midi, red ring). Attach the assembled column stack onto a port of the vacuum manifold tightly.**
   Most commercial vacuum manifold with luer connectors can be used.

2. **Decant all of the lysate to EzClear™ Filter (Midi, blue ring) unit and incubate 1~3 min to allow the cellular debris and precipitates to rise to the top.**

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

4. **Discard the upper EzClear™ Filter (Midi, blue ring) and apply 9 ml of Buffer AW to Column Type Q (Midi, red ring). Switch on vacuum source to draw the solution through the column and slowly release the vacuum.**
   This step will remove any traces of proteins, carbohydrates, and other cellular components bound nonspecifically to the column membrane.

5. **Apply 14 ml of Buffer PW and switch on vacuum source. When all liquid has been pulled through the Column Type Q (Midi, red ring), slowly release the vacuum.**

6. **Transfer the Column Type Q (Midi, red ring) to a collection tube (provided).**

7. **Go to step 5 in 'Centrifugation Protocol' (page 24).**

Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low or no yield of plasmid DNA	**Too many cells in sample**	Cultures should be grown for 16~21 hours in proper media with antibiotics. Reduce the 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).
	Low-copy-number plasmid used	Low-copy-number plasmid may yield as little as 0.5 μg 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 must be thoroughly resuspended in Buffer S1.
	Buffer S2 precipitated	Redissolve Buffer S2 by warming at 37°C (or above).
	Insufficient digestion with RNase A	Excess RNA can interfere the binding of plasmid DNA with Column Type Q membrane. Store Buffer S1 at 4°C after the addition of RNase A. If Buffer S1 containing RNase A is more than a year old, the activity of RNase A can be decreased slightly.
	Inadequate elution buffer	DNA is eluted only in low salt condition. Buffer EB (10 mM 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.

Facts	Possible Causes	Suggestions
	Improper centrifuge (Midi)	Swinging-bucket rotor (capable of 4,000~5,000 x g) should be used. Use of fixed-angle rotor may lead to failure of proper contact between the lysate and the Column Type Q membrane resulting in poor and inconsistent yield of DNA.
Low purity	**Contamination of precipitate when binding**	When the cleared lysate is transferred to Column Type Q, ensure that any precipitate does not contain to the transfer.
	Improper centrifuge (Midi)	Swinging-bucket rotor (capable of 4,000~5,000 x g) should be used instead of fixed angle rotor.
Chromosomal DNA contamination	**Mis-handling of the lysate after addition of Buffer S3**	Vigorous vortexing after addition of Buffer S3 can cause shearing of chromosomal DNA followed by chromosomal DNA contamination. Handle gently the lysate after addition of Buffer S3. Simple inverting and rotating tube to cover walls with lysate is sufficient for mixing.
Smearing of plasmid DNA	**Too long lysis time**	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.
	Vigorous mixing in Buffer S2	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.
Lysate filtered by EzClear™ Filter is not clear	**Excessive salt-precipitates in lysate (Midi)**	The biomass in starting sample is small. Decrease the volume of buffers during alkaline lysis. Otherwise, increase the amount of starting sample.

Facts	Possible Causes	Suggestions
RNA Contamination	**RNase A omitted or old**	RNase A should be added to Buffer S1 before first use. If Buffer S1 containing RNase A is more than a year old, the activity of RNase A can be decreased. Add additional RNase A (working concentration=100 μg/ml). Buffer S1 containing RNase A 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 A digestion.
High salt concentration in eluate	**Improper wash step**	Ensure the wash step in protocol. Alternatively, incubate for 5 min at room temperature after applying Buffer PW in wash step.
Plasmid DNA degradation	**Nuclease contamination**	For *endA*$^+$ strains such as HB101 and the JM series (page 13), washing with Buffer AW should be carried out properly.
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. Column Type Q 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.
	Low purity of DNA	See"Low purity" at page 29.
	Residual ethanol in eluate	Ensure that the washing steps are performed properly. Column Type Q membrane should be completely dried via additional centrifugation or air-drying.

Exprep™ Plasmid SV mini

1. Pellet cells by centrifugation
2. Resuspend in 250 µl of Buffer S1
3. Add 250 µl of Buffer S2 and mix by inverting
4. Add 350 µl of Buffer S3 and mix by inverting
5. Centrifuge for 10 min
6. Transfer the cleared lysate to Column Type Q (mini) and centrifuge for 30 sec
7. *(Optional:)* Add 500 µl of Buffer AW and centrifuge for 30 sec
8. Add 700 µl of Buffer PW and centrifuge for 30 sec
9. Centrifuge for additional 1 min
10. Apply 50 µl of Buffer EB and centrifuge for 1 min

Exprep™ Plasmid SV Midi

1. Pellet cells by centrifugation
2. Resuspend in 2.5 ml of Buffer S1
3. Add 2.5 ml of Buffer S2 and mix by inverting
4. Add 3.5 ml of Buffer S3 and mix by inverting
5. *(Optional:)* Centrifuge for 20 min at 4,500 x g (5,000 rpm)
6. Transfer the lysate (step 4) or the cleared lysate (step 5) to EzClear™ Filter (Midi, blue ring), let stand for 2 min and centrifuge for 3 min at 1,000 x g (2,200 rpm)
7. Transfer the pass-through to Column Type Q (Midi, red ring) and centrifuge for 3 min at 1,000 x g (2,200 rpm)
8. Add 9 ml of Buffer AW and centrifuge for 3 min at 1,000 x g
9. Add 12 ml of Buffer PW and centrifuge for 3 min at 1,000 x g
10. Add 3 ml of Buffer PW and centrifuge for 15 min at 4,500 x g
11. Apply 600 µl of Buffer EB, let stand for 5 min and centrifuge for 5 min at 4,500 x g

Ordering Information

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

Products	Scale	Size	Cat. No.	Type
Plasmid Rapidprep	mini	50	100-150	spin
		200	100-102	

GeneAll® *Exprep™* *for preparation of plasmid DNA*

Products	Scale	Size	Cat. No.	Type
Plasmid SV	mini	50	101-150	spin / vacuum
		200	101-102	
	Midi	26	101-226	spin / vacuum
		50	101-250	
		100	101-201	

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

Products	Scale	Size	Cat. No.	Type
Plasmid LE *(Low Endotoxin)*	mini	50	111-150	spin / vacuum
		200	111-102	
	Midi	26	111-226	spin / vacuum
		100	111-201	
Plasmid EF *(Endotoxin Free)*	Midi	20	121-220	spin
		100	121-201	

GeneAll® *Expin™* *for purification of fragment DNA*

Products	Scale	Size	Cat. No.	Type
Gel SV	mini	50	102-150	spin / vacuum
		200	102-102	
PCR SV	mini	50	103-150	spin / vacuum
		200	103-102	
CleanUp SV	mini	50	113-150	spin / vacuum
		200	113-102	
Combo GP	mini	50	112-150	spin / vacuum
		200	112-102	

GeneAll® *Exgene™* *for isolation of total DNA*

Products	Scale	Size	Cat. No.	Type
Tissue SV	mini	100	104-101	spin / vacuum
		250	104-152	
	Midi	26	104-226	spin / vacuum
		100	104-201	
	MAXI	10	104-310	spin / vacuum
		26	104-326	
Tissue Plus SV	mini	100	109-101	spin / vacuum
		250	109-152	
	Midi	26	109-226	spin / vacuum
		100	109-201	
	MAXI	10	109-310	spin / vacuum
		26	109-326	

GeneAll® *Exgene™* *for isolation of total DNA*

Products	Scale	Size	Cat. No.	Type
Blood SV	mini	100	105-101	spin / vacuum
		250	105-152	
	Midi	26	105-226	spin / vacuum
		100	105-201	
	MAXI	10	105-310	spin / vacuum
		26	105-326	
Cell SV	mini	100	106-101	spin / vacuum
		250	106-152	
	MAXI	10	106-310	spin / vacuum
		26	106-326	
Clinic SV	mini	100	108-101	spin / vacuum
		250	108-152	
	Midi	26	108-226	spin / vacuum
		100	108-201	
	MAXI	10	108-310	spin / vacuum
		26	108-326	
Genomic DNA micro		50	118-050	spin
Plant SV	mini	100	117-101	spin / vacuum
		250	117-152	
	Midi	26	117-226	spin / vacuum
		100	117-201	
	MAXI	10	117-310	spin / vacuum
		26	117-326	
Soil DNA mini	mini	50	114-150	spin
Stool DNA mini	mini	50	115-150	spin
Stool-Bead DNA mini	mini	50	115-151	spin
Viral DNA/RNA	mini	50	128-150	spin
FFPE Tissue DNA	mini	50	138-150	spin
		250	138-152	
Forensic	mini	100	122-101	spin / vacuum
		250	122-152	
cfDNA	mini	100	129-101	spin / vacuum

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

Products	Scale	Size	Cat. No.	Type
GenEx™ Blood	Sx	100	220-101	solution
		500	220-105	
	Lx	100	220-301	solution
GenEx™ Cell	Sx	100	221-101	solution
		500	221-105	
	Lx	100	221-301	solution
GenEx™ Tissue	Sx	100	222-101	solution
		500	222-105	
	Lx	100	222-301	solution

Products	Scale	Size	Cat. No.	Type
GeneAll® *GenEx™* *for isolation of total DNA without spin column*				
GenEx™ Plant	Sx	100	227-101	solution
	Mx	100	227-201	
	Lx	100	227-301	
GenEx™ Plant Plus	Sx	100	228-101	solution
	Mx	50	228-250	
	Lx	20	228-320	
GeneAll® *DirEx™* series *for preperation of PCR-template without extraction*				
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	260-031	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 preperation of total RNA*				
RiboEx™	mini	100	301-001	solution
		200	301-002	
Hybrid-R™	mini	100	305-101	spin
Hybrid-R™ Blood RNA	mini	50	315-150	spin
Hybrid-R™ miRNA	mini	50	325-150	spin
RiboEx™ LS	mini	100	302-001	solution
		200	302-002	
Riboclear™	mini	50	303-150	spin
Riboclear™ Plus	mini	50	313-150	spin
Ribospin™	mini	50	304-150	spin
Ribospin™ II	mini	50	314-150	spin
		300	314-103	
Ribospin ™ vRD	mini	50	302-150	spin
Ribospin ™ vRD 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™ Pathogen/TNA	mini	50	314-150	spin
		250	314-152	
Allspin™	mini	50	306-150	spin
RiboSaver™	mini	100	351- 001	solution

Products	Scale	Size	Cat. No.	Type
GeneAll® *AmpONE™* *for PCR amplification*				
Taq DNA polymerase		250 U	501-025	(2.5 U/μl)
		500 U	501-050	
		1,000 U	501-100	
Taq Premix	20 μl x 96 tubes		526-200	solution
	50 μl x 96 tubes		526-500	
GeneAll® *AmpMaster™* *for PCR amplification*				
Taq Master mix	0.5 ml x 2 tubes		541-010	solution
	0.5 ml x 10 tubes		541-050	solution
GeneAll® *HyperScript™* *for Reverse Transcription*				
Reverse Transcriptase	10,000 U		601-100	solution
RT Master mix	0.5 ml x 2 tubes		601-710	solution
One-step RT-PCR Master mix	0.5 ml x 2 tubes		602-110	solution
One-step RT-PCR Premix	20 μl x 96 tubes		602-102	solution
GeneAll® RealAmp™ *for qPCR amplification*				
SYBR qPCR Master mix (2X, Low ROX)	200 rxn	2 ml	801-020	solution
	500 rxn	5 ml	801-050	
SYBR qPCR Master mix (2X, High ROX)	200 rxn	2 ml	801-021	solution
	500 rxn	5 ml	801-051	
GeneAll® Protein series				
ProtinEx™ Animal cell/tissue	100 ml		701-001	solution
PAGESTA™ Reducing 5X SDS-PAGE Sample Buffer	1 ml x 10 tubes		751-001	solution

Products	Size	Cat. No.	Type
GeneAll® GENTi™ 32 ADVANCED *Newly designed automated extraction system*			
Automatic extraction equipment		GTI032A	system
Genomic DNA	48	901-048A	tube
	96	901-096A	plate
Viral DNA/RNA	48	902-048A	tube
	96	902-096A	plate
Blood DNA	48	903-048A	tube
	96	903-096A	plate
Plant DNA/RNA	48	904-048A	tube
	96	904-096A	plate
LMO	48	906-048A	tube
	96	906-096A	plate
Fecal DNA/RNA	48	913-048A	tube
	96	913-096A	plate
Forensic DNA	48	914-048A	tube
	96	914-096A	plate
Cell/Tissue Total RNA	48	915-048A	tube
	96	915-096A	plate
Plant Total RNA	48	916-048A	tube
	96	916-096A	plate
cfDNA	48	917-048A	tube
	96	917-096A	plate

Products	Size	Cat. No.	Type
GeneAll® AllEx®64 *Compact yet Comprehensive automated extraction system*			
Automatic extraction equipment		AEX064	system
Genomic DNA	48	931-048	single
	96	931-096	plate
Viral DNA/RNA	48	934-048	single
	96	934-096	plate
Blood DNA	48	935-048	single
	96	935-096	plate
Plant DNA/RNA	48	937-048	single
	96	937-096	plate
Fecal DNA/RNA	48	948-048	single
	96	948-096	plate
Forensic	48	936-048	single
	96	936-096	plate
Cell/Tissue Total RNA	48	951-048	single
	96	951-096	plate
Plant Total RNA	48	952-048	single
	96	952-096	plate
cfDNA	48	953-048	single
	96	953-096	plate

GeneAll® AllEx®Mini *Compact yet Comprehensive automated extraction system*			
Automatic extraction equipment		AEX012	system
Genomic DNA	48	971-048	single
Viral DNA/RNA	48	972-048	single
Blood DNA	48	973-048	single
Plant DNA/RNA	48	974-048	single
Forensic	48	975-048	single
Fecal DNA/RNA	48	976-048	single
Cell/Tissue Total RNA	48	977-048	single
Plant Total RNA	48	978-048	single

Note

Note

GeneAll

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