Ver 3.0

Handbook for

Hybrid-Q™

Plasmid Rapidprep

DNA PURIFICATION KIT HANDBOOK

hybrid-Q tm

GeneAll

Customer & Technical Support

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

We appreciate your comments and advice.

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This protocol handbook is included in :

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

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

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	Hybrid-Q™	
Cat. No.	**100-150**	**100-102**
No. of preparations	50	200
Column Type Q (with collection tube)	50	200
EzClear™ Filter (with collection tube)	50	200
Buffer S1	20	60 ml
Buffer S2	20	60 ml
Buffer G3	25	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 μg	300 μg
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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*

Precautions and Disclaimer

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

Chemical Hazard

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

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

Quality Control

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

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

Storage Conditions

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

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

Product Specifications

Hybrid-Q™ Plasmid Rapidprep	
Size	**mini**
Format	Spin
Recommended sample volume (High copy)	2~5 ml
Maximum sample volume (Low copy)	10 ml
Maximum loading volume of EzClear™ Filter	600 μl
Maximum loading volume of Column Type Q	800 μl
Binding capacity	30 μg
Recovery rate	85~95%
Minimum elution volume	40 μl

Hybrid-Q™ Plasmid Rapidprep Kit

Introduction

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

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

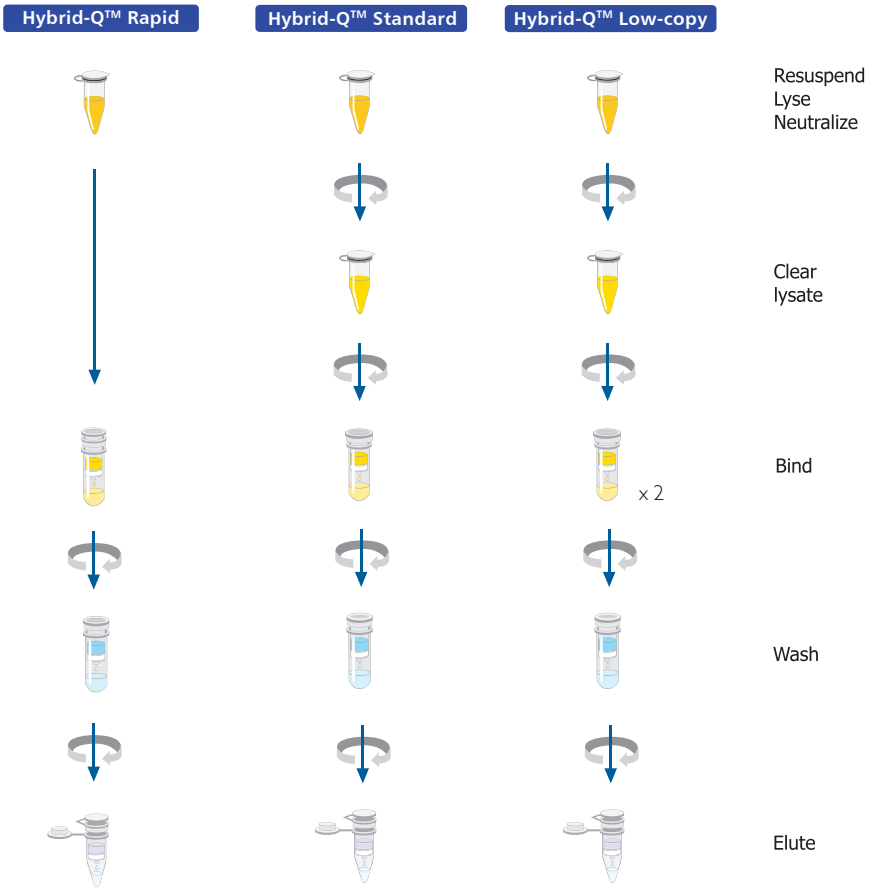
Up to 30 μg of pure plasmid can be purified using Hybrid-Q™ Plasmid Rapidprep Kit and this pure plasmid DNA is ready for PCR, cloning, fluorescent sequencing, synthesis of labeled hybridization probes, cell transfection, electroporation, and enzymatic restriction analysis without further manipulation.

Principle of Method

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

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

Hybrid-Q™ Plasmid Rapidprep
Quick View



High-purity plasmid DNA

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.
Whenever possible, plasmids should be purified from bacterial cultures that have been inoculated with a single transformed colony picked from an agar plate.
Usually, the colony is transferred to a small starter culture, which is grown to late log phase. Aliquots of this culture can be used to prepare small amounts of the plasmid DNA for analysis and/or as the inoculum for a large-scale culture. The conditions of growth of the large-scale culture depend chiefly on the copy number of the plasmid and whether it replicates in a stringent or relaxed fashion. At all times, the transformed bacteria should be grown in selective conditions, i.e., in the presence of the appropriate antibiotics.
The copy number of a plasmid is defined as the average number of plasmids per bacterial cells under normal growth conditions. Plasmids have own copy number per cell, depending on their origin of replication (replicon) and the size of plasmid DNA. A plasmid replicon can be defined as the smallest piece of plasmid DNA that is able to replicate autonomously and maintain normal copy number by determining whether they are under relaxed or stringent control.

More than 30 different replicons have been identified in plasmids. However, almost all plasmids used routinely in molecular cloning carry a replicon derived from pMB1. pUC plasmids contain a modified pMB1 replicon, have relaxed control, and replicate to a very high copy number, otherwise pSC101 has stringent control and maintain low-copy number. Generally, high-copy number plasmid will result in higher yield.
Very large plasmids are often maintained at very low copy numbers per cell.
Hybrid-Q™ Plasmid Rapidprep Kit Procedure is optimized to high-copy number plasmid, so more starting sample may be needed if low-copy number plasmids are used.

Table 1. Replicons carried by various plasmid vectors

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

Most *E.coli* strains can be used to propagate and isolate plasmid DNA. Host strains such as 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 12). If endonuclease I is not completely removed during plasmid preparations, the plasmid DNA in eluate is degraded during subsequent incubation in the presence of Mg^{2+} (e.g. during PCR or the incubation with restriction enzyme). This problem can be avoided by use of *endA*$^-$ strains (denoted as *endA1*) such as DH5α and XL1-Blue. Extra wash with Buffer AW will also help prevent the degradation of DNA.

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

Alkaline lysis

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

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

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

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

Filtration of lysate with EzClear™ Filter

After mixing with Buffer G3, 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.

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

Washing

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

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

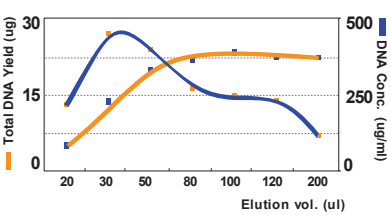
Table 2. The genotype of various *E.coli* strains

***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 depending on the need for downstream applications. Buffer EB contains 10 mM TrisCl, pH 8.5. When using water for eluent, make sure that the pH value is within 7.0 and 8.5.

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



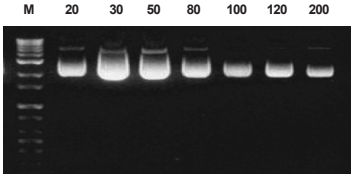


Figure 1. The overall yield and concentration of plasmid DNA depending on the volume of elution. pUC18 plasmid DNA was purified from 3 ml of overnight cultured DH5α using Hybrid-Q™ Plasmid Rapidprep Kit. Plasmid DNA was eluted with the indicated volume of Buffer EB, and resolved on 1% agarose gel.

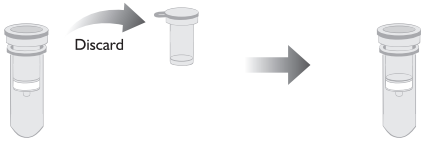
How to use column

1. Rapid protocol

Use Ezclear™ Filter (purple) + Column Type Q (yellow) + collection tube

2. Standard/Low-copy protocol

Discard Ezclear™ Filter (purple) Use Column Type Q (yellow) + collecion tube



Brief Protocol

Hybrid-Q™

Rapid Protocol

1. Pellet 1~3 ml of culture by centrifugation
2. Resuspend in 170 μl of Buffer S1
3. Add 170 μl of Buffer S2 and mix by inverting
4. Add 250 μl of Buffer G3 and mix by inverting
5. Transfer the lysate to EzClear™ Filter stack by decanting
6. Centrifuge for 30 sec and discard the EzClear™ Filter (upper, violet)
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

Hybrid-Q™ Rapid Protocol

Before experiment

Unless there is 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 solution into Buffer S1 before first use and store it at 4°C.

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

Prepare new 1.5 ml or 2.0 ml microcentrifuge tubes.

1. Pellet 1~3 ml of the bacterial culture by centrifugation for 1 min at 13,000 x g. Discard the supernatant as much as possible.

Use the appropriate volume of bacterial cultures; too much amount of starting sample can clog the EzClear™ Filter. Bacterial culture should be grown for 16 to 21 hours in LB media containing a selective antibiotic. Use of other rich broth, such as TB or 2xYT, and/or higher culture volume can cause reduction of lysis efficiency, clogging of EzClear™ Filter or overload of a Column Type Q, resulting in unsatisfactory yield. For more than 2 ml of overnight culture, bacterial cells can be collected in 15 ml conical tube by centrifugation for 5 min at 10,000 x g in a tabletop centrifuge. Alternatively, bactrial cells can be collected repeatedly in 1.5 ml or 2.0 ml microcentrifuge tube.

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

It is essential to thoroughly resuspend the cell pellet.

* Add RNase A solution into Buffer S1 before the first use.

You don't need to transfer the suspension if the tube used for pelleting is an 1.5 ml microcentrifuge tube.

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

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

   If precipitated material has formed in Buffer S2, heat to dissolve at 37°C. Precipitated Buffer S2 may cause significant decrease in the recovery yield of DNA.

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

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

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

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

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

6. ***(Optional :)*** **Apply 500 µl of Buffer AW and centrifuge for 30 sec. Remove the Column Type Q, 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 *endA1* refers to a mutation in the wildtype *endA* gene, which produces an inactive form of the nuclease. *E.coli* strains with this mutation are referred to as *endA*$^-$.

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

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

7. **Apply 700 µl of Buffer PW and centrifuge for 30 sec. Remove the Column Type Q, discard the pass-through, and re-insert the column to the collection tube.**

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

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

9. Add 50 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 membrane for optimal elution of DNA.

Eluent volume can be adjusted to 100~200 μl and it will increase the total yields 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 value of water is between 7.0 and 8.5.

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

Brief Protocol

Hybrid-Q™
Standard Protocol

1. Pellet up to 5 ml of culture 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 G3 and mix by inverting
5. Centrifuge for 10 min
6. Transfer the cleared lysate to Column Type Q 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

Hybrid-Q™
Standard Protocol

Before experiment

Unless there is 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 solution into Buffer S1 before first use and store it at 4°C.

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

Prepare new 1.5 ml or 2.0 ml microcentrifuge tubes.

1. Pellet up to 5 ml of the bacterial culture by centrifugation for 5 min at 10,000 x g. Discard the supernatant as much as possible.

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

Alternatively, bacterial cells can be pelleted repeatedly in 1.5 ml or 2.0 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.

* Add RNase A solution into Buffer S1 before first use.

You don't need to transfer the suspension if the tube used for pelleting is an 1.5 ml microcentrifuge tube.

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 the recovery yield of DNA.

4. **Add 350 μl 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.
   Vigorous handling may lead to the contamination of genomic DNA and the decrease in quality of plasmid DNA.

5. **Centrifuge for 10 min.**

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

7. ***(Optional :)*** **Apply 500 µl of Buffer AW and centrifuge for 30 sec. Remove the Column Type Q, discard the pass-through, and reinsert 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 *endA1* refers to a mutation in the wildtype *endA* gene, which produces an inactive form of the nuclease. *E.coli* strains with this mutation are referred to as *endA*$^-$.

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

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

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

9. **Centrifuge for an additional 1 min to remove residual wash buffer. Transfer the Column Type Q 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.

10. 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 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.

Brief Protocol

Hybrid-Q™

Low-copy plasmid Protocol

1. Pellet up to 10 ml of culture into 2.0 ml microcentrifuge tube by centrifugation
2. Resuspend in 400 µl of Buffer S1
3. Add 400 µl of Buffer S2 and mix by inverting
4. Add 600 µl of Buffer G3 and mix by inverting
5. Centrifuge for 10 min
6. Transfer 700 µl of the cleared lysate to Column Type Q and centrifuge for 30 sec
7. Transfer the remainder into the column and centrifuge for 30 sec
8. Add 500 µl of Buffer AW and centrifuge for 30 sec
9. Add 700 µl of Buffer PW and centrifuge for 30 sec
10. Centrifuge for additional 1 min
11. Apply 50 µl of Buffer EB and centrifuge for 1 min

Hybrid-Q™
Low-copy plasmid Protocol

Before experiment

Unless there is 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 solution into Buffer S1 before first use and store it at 4°C.

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

Prepare new 1.5 ml or 2.0 ml microcentrifuge tubes.

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

1. Pellet up to 10 ml of the bacterial culture by centrifugation for 5 min at 10,000 x g. Discard the supernatant as much as possible.

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

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

Alternatively, bacterial cells can be pelleted repeatedly into a 2.0 ml microcentrifuge tube by centrifugation for 1 min at full speed.

2. Resuspend pelleted bacterial cells thoroughly in 400 μl of Buffer S1. Transfer the suspension to a new 2.0 ml microcentrifuge tube.

It is essential to thoroughly resuspend the cell pellet.

* Add RNase A solution into Buffer S1 before first use.

3. **Add 400 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 the recovery yield of DNA.

4. **Add 600 μl of Buffer G3 and immediately mix by inverting the tube 4~6 times (DO NOT VORTEX).**
   For better precipitation, mix the lysate gently but completely and immediately after addition of Buffer G3.

5. **Centrifuge for 10 min. Transfer carefully the supernatant to a new 2.0 ml microcentrifuge tube by decanting or pipetting.**
   Avoid the white precipitate cotransfering into a new tube.

6. **Transfer 700 μl of the cleared lysate into a Column Type Q. Centrifuge for 30 sec. Remove the column, discard the pass-through, and re-insert the column to the collection tube.**

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

8. Apply 500 μl of Buffer AW and centrifuge for 30 sec. Remove the Column Type Q, discard the pass-through, and reinsert 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 *endA1* refers to a mutation in the wildtype *endA* gene, which produces an inactive form of the nuclease. *E.coli* strains with this mutation are referred to as *endA*$^-$.

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

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

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

10. Centrifuge for an additional 1 min to remove residual wash buffer. Transfer the Column Type Q 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.

11. 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 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.

Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low or no yield of plasmid DNA	**Poor lysis due to too many cells in the sample.**	Cultures should be grown for 16~21 hours in proper media with antibiotics. Reduce the volume of sample.
	Low-copy-number plasmid used	Low-copy-number plasmid may yield as little as 0.5 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 should be thoroughly resuspended in Buffer S1.
	Buffer S2 precipitated	Redissolve Buffer S2 by warming at 37°C or above.
	Insufficient digestion with RNase	Excess RNA can interfere the binding of plasmid DNA with 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.
	Inadequate elution buffer	DNA can be 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.
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.

Facts	Possible Causes	Suggestions
Chromosomal DNA contamination	**Mis-handling of the lysate after addition of Buffer G3**	Vigorous vortexing after addition of Buffer G3 can cause shearing of chromosomal DNA followed by chromosomal DNA contamination. Handle gently the lysate after addition of Buffer G3. Simple inverting and rotating tube to cover walls with lysate is sufficient for mixing.
	Too large sample	Reduce the sample volumes.
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.
EzClear™ Filter clogging	**Too many cells in the sample**	Reduce the sample volume.
RNA Contamination	**RNase 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.

Facts	Possible Causes	Suggestions
High salt concentration in eluate	**Improper wash step**	Ensure that washing steps are performed properly. Alternatively, incubate for 5 min at room temperature after applying Buffer PW in wash step.
Plasmid DNA degradation	**Nuclease contamination**	For *endA*$^+$ strains such as HB101 and the JM series, washing with Buffer AW should be carried out properly. Refer to page 12.
DNA floats out of well while loading of agarose gel	**Ethanol is not completely removed during wash steps**	Ensure that washing steps are performed properly. 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.
	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.

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	

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
GeneAll® AllEx®64 *Compact yet Comprehensive automated extraction system*			
Automatic extraction equipment		AEX064	system
Genomic DNA	48	931-048A	tube
	96	931-096A	plate
Viral DNA/RNA	48	934-048A	tube
	96	934-096A	plate
Blood DNA	48	935-048A	tube
	96	935-096A	plate
Plant DNA/RNA	48	937-048A	tube
	96	937-096A	plate
Fecal DNA/RNA	48	948-048A	tube
	96	948-096A	plate

Note

GeneAll

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