Kiagen Nucleic Acid Purification Kits:

Filter based Kits

- KiaSpin® PCR Template Purification Kit
- KiaSpin® Viral Nucleic Acid Purification Kit
- KiaSpin® PCR Product Purification Kit
- KiaSpin® Gel Extraction Kit
- KiaSpin® Genomic DNA Purification Kit
- KiaSpin® RNA Purification Kit
- KiaSpin® Viral RNA Purification Kit

Sorbant based Kits

- KiaSorb® PCR Template Purification Kit
- KiaSorb® Viral Nucleic Acid Purification Kit
- KiaSorb® PCR Product Purification Kit
- KiaSorb® Gel Extraction Kit
- KiaSorb® Genomic DNA Purification Kit
- KiaSorb® RNA Purification Kit

Address:
Kiagen Teb Co.
Unit 31, 10th Flour, No 137, Dogol Tower, Opposite of Amir-Atabak
Motahari St, Tehran
Tel: 021-88760179, 021-22648647, 0912-3847794, 0939 -1125991
Fax: 021-88759622
www.kiagen-biotech.com info@kiagen-biotech.com

KiaSpin® Plasmid Extraction kit

for recovery of up to 100 samples

Trouble shooting

Problem	Possible Cause	Recommendation
Low nucleic acid yield or purity	Kit stored under non-optimal conditions	Store kit at 15-25°C at all times upon arrival.
	Buffers or other reagents were exposed to conditions that reduce their effectiveness.	• Store all buffers at 15-25°C. • Close all reagent bottles tightly after each use to preserve pH, stability, and freedom from contamination.
	Reagents and samples not completely mixed	Always mix the sample tube well after addition of each reagent.
Low recovery of nucleic acids after elution	Non-optimal reagent has been used for elution. Alkaline pH is required for optimal elution.	• Do not use water to elute nucleic acids from sorbant. • Use the Elution Buffer in the kit.
Incomplete or no PCR or restriction enzyme cleavage of product	Silica membrane which can coelute with the nucleic acid may inhibit enzyme reactions	• After elution step is complete, remove silica membrane from tube containing eluted with spin this sample tube for 1 minute at maximum speed. • Transfer supernatant into a new tube without disturbing the silica membrane at the bottom of the original tube.
	Eluate containing the purified DNA product is contaminated with ethanol from the Wash Buffer.	After the 2rd wash step dry the silica membrane completely
Absorbency (A_{260}) reading of product too high	Silica membrane which can coelute with nucleic acid.	See suggestions under "Incomplete or no restriction enzyme cleavage of product" above.
Sample "pop" out of wells in agarose gels	Eluate containing the purified DNA product is contaminated with ethanol from the Wash Buffer.	After the 2rd wash step dry the silica membrane completely.

.

Kit contents

KiaSpin® Plasmid Extract Kit		
Cat No.	**50 preps**	**100 preps**
RNase A	5 mg	10 mg
Spin Column	50	100
Collection tubes	50	100
Buffer P1 (Resuspension Buffer)	20 ml	25 ml
Buffer P2 (Lysis Buffer)	20 ml	25 ml
Buffer N3 (Neutralization & Binding Buffer)	25 ml	50 ml
Buffer IRB (Inhibitor Removal Buffer)	30 ml	60 ml
Buffer WB (Wash Buffer)	50 ml	100 ml
Buffer EB (Elution Buffer)	15 ml	30 ml

- All solutions are clear, and not to be used when precipitates have formed.
- If precipitates have formed simply warm the solution at 15-25°C or in a 37°C water bath until the precipitates have dissolved before using.
- The binding buffer contains irradiants which is harmful when either in contact with skin, or when inhaled or swallowed. Always wear gloves, and follow standard safety precautions during handling.

Introduction

The KiaSpin® Miniprep system provides a fast, simple, and cost-effective plasmid miniprep method for routine molecular biology laboratory applications. KiaSpin® Miniprep Kits use silica membrane technology to eliminate the cumbersome steps associated with loose resins or slurries. Plasmid DNA purified with KiaSpin® Miniprep Kits is immediately ready for use. Phenol extraction and ethanol precipitation are not required, and high-quality plasmid DNA is eluted in a small volume of Tris buffer (included in each kit) or water. The KiaSpin® system consists of four products with different handling options to suit every throughput need.

Applications

Plasmid DNA prepared using the KiaSpin system is suitable for a variety of routine applications including:

- Restriction enzyme digestion
- Library screening
- In vitro translation
- Sequencing
- Ligation and transformation
- Transfection of robust cells

Storage conditions and preparation of working solutions

KiaSpin® Miniprep Kits should be stored dry at room temperature (15-25°C). Kits can be stored for up to 12 months without showing any reduction in performance and quality. For longer storage these kits can be kept at 2-8°c. If any precipitate forms in the buffers after storage at 2-8°C it should be redissolved by warming the buffers to 37°C before use. After addition of RNase A, Buffer P1 is stable for six months when stored at 2-8°c. RNase A stock solution can be stored for two years at -20°c.

Kit Specifications

DNA binding capacity	10-40 µg
Recovery	80-90%
Elution volume	50-100 µl

Elution procedure

- For the elution of DNA one of the following solutions can be used:
  Buffer EB (supplied) / TE buffer, pH 8.5/ Distilled water, pH 8.5

- Note: EDTA in TE buffer may cause problems in subsequent reactions, and the pH of distilled water should be checked before use to avoid lower recovery yields.

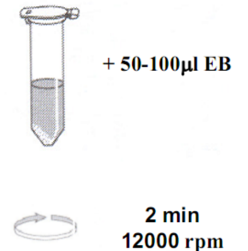
- KiaSpin® Extract: With an elution volume of 50 µl of buffer EB typical recovery rates of 70-90% are regularly obtained for plasmids between 2-10 kb.

IMPORTANT: Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer WB may inhibit subsequent enzymatic reactions

8. Elute DNA

Place the KiaSpin® Extract column into a clean 1.5 ml microcentrifuge tube.

Add 50-100 µl elution buffer EB, incubate at room temperature for 1 min to increase the yield of eluted DNA. Centrifuge for 1-2 min at 12,000 rpm.



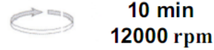
Yield of larger fragments (> 5-10 kb) can be increased by using pre-warmed elution buffer (65 °C): For elution, add pre-warmed elution buffer and incubate at room temperature for 2 min before collecting eluate by centrifugation.

Determination of yield

To determine the yield, DNA concentration should be determined by both UV spectrophotometry and quantitative analysis on an agarose gel.

The lysate should be mixed thoroughly to ensure even potassium dodecyl sulfate precipitation. If the mixture still appears viscous and brownish, more mixing is required to completely neutralize the solution.

6. Centrifuge 10 minute at 12000-14000 rpm.



10 min
12000 rpm

A compact pellet will form.

During centrifugation, place a spin column in a 2 ml collection tube.

7. Bind DNA

Apply the supernatants from step 5 to the spin column by decanting or pipetting.

Centrifuge 30-60 s. Discard the flow-through and place the KiaSpin® column back into the collecting tube.

30-60 sec
12000 rpm

(Optional): Wash spin column by adding 0.5 ml of Buffer IRB and centrifuging 30-60 s. Discard the flow-through.

This step is necessary to remove trace nuclease activity when using endA+ strains such as the JM series, HB 101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XI-1 Blue and DH5α do not require this additional wash step.

7.Wash silica membrane

1st wash

Wash spin column by adding 0.6 ml of buffer WB and centrifuging 30-60 s.

Discard the flow-through.

1st 600 μl WB
2nd 400 μl WB

2nd wash

Wash spin column by adding **0.4 ml** of buffer WB and centrifuging 30-60 s.

30-60 sec
12000 rpm

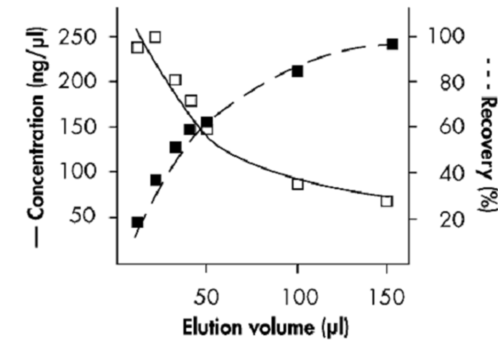
Discard the flow-through and centrifuge for an additional 1 min to remove residual wash buffer.

1 min
12000 rpm

DNA yield

Plasmid yield with the KiaSpin miniprep system varies depending on plasmid copy number per cell, the individual insert in a plasmid, factors that affect growth of the bacterial culture (see pages 5-8), the elution volume, and the elution incubation time. A 1.5 ml overnight culture can yield from 5 to 15 µg of plasmid DNA. To obtain the optimum combination of DNA quality, yield, and concentration, we recommend using Luria-Bertani (LB) medium for growth of cultures, eluting plasmid DNA in a volume of 50 µl, and performing a short incubation after addition of the elution buffer.

Elution Volume versus DNA Concentration and Recovery



The KiaSpin® Plasmid Extract Principle

The **KiaSpin® Plasmid Extract** miniprep procedure is based on alkaline lysis of bacterial cells followed by adsorption of DNA onto silica membrane in the presence of high salt.

The procedure consists of three basic steps:

- preparation and clearing of a bacterial lysate
- adsorption of DNA onto the Silica membrane
- washing and elution of plasmid DNA

All steps are performed without the use of phenol, chloroform, CsCl, ethidium bromide, and without alcohol precipitation.

Growth of bacterial cultures

Plasmids are generally prepared from bacterial cultures grown in the presence of a selective agent such as an antibiotic. The yield and quality of the plasmid DNA prepared may depend on a number of factors including plasmid copy number, size of insert, host strain, culture volume, and culture medium.

Plasmid copy number

Plasmids vary widely in their copy number (Table 1), depending on the origin of replication they contain (pMB1 or pSC101 for example) which determines whether they are under relaxed or stringent control; as well as the size of the plasmid and its associated insert. Some plasmids, such as the pUC series and derivatives, have mutations which allow them to reach very high copy numbers within the bacterial cell.

Plasmids based on pBR322 and many cosmids are generally maintained at lower copy numbers. Very large plasmids are often maintained at very low copy numbers per cell.

Please note: The copy number of plasmids and cosmids can be substantially influenced by the cloned insert. For example, a high-copy pUC plasmid may behave like a medium or low-copy plasmid when containing certain inserts (e.g., very large DNA fragments), resulting in lower DNA yields than expected.

Table 1: Origins of replication and copy number of various plasmids and cosmids

DNA construct	Origin of replication	Copy number	Classification
Plasmids			
pUC vectors	pMB1*	500–700	high copy
pBluescript® vectors	ColE1	300–500	high copy
pGEM® vectors	pMB1*	300–400	high copy
pTZ vectors	pMB1*	>1000	high copy
pBR322 and derivatives	pMB1*	15–20	low copy
pACYC and derivatives	p15A	10–12	low copy
pSC101 and derivatives	pSC101	~5	very low copy
Cosmids			
SuperCos	pMB1	10–20	low copy
pWE15	ColE1	10–20	low copy

** The pMB1 origin of replication is closely related to that of ColE1 and falls in the same incompatibility group. The high-copy plasmids listed here contain mutated versions of this origin.*

Procedure

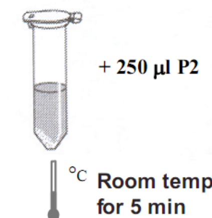
1. **Pick a single colony from a freshly streaked selective plate and inoculate 2–5 ml P2 medium containing the appropriate selective antibiotic. Incubate for 12–16 h at 37°C with vigorous shaking (150-300 rpm).**
   Use a tube or flask with a volume of at least 4 times the volume of the culture.

2. **Harvest the bacterial cells by centrifugation at 6000 x g for 15 min at 4°C.**
   If you wish to stop the protocol and continue later, freeze the cell pellets at –20°C.

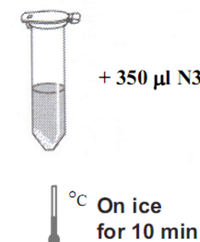
3. **Resuspend the bacterial pellet in 250 µl of Buffer P1.**
   Ensure that RNase A has been added to Buffer P1.
   The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

4. **Add 250µl Buffer P2, mix gently but thoroughly by inverting 4–6 times and incubate at room temperature for 5 min.**
   Do not vortex, as this will result in shearing of genomic DNA.
   The lysate should appear viscous.

Note: Do not allow the lysis reaction to proceed for more than 5 min. After use, the bottle containing Buffer P2 should be closed immediately to avoid acidification from CO_2 in the air.



5. **Add 350µl of chilled Buffer N3, mix immediately but gently by inverting 4–6 times, and incubate on ice for 10 min.**
   Precipitation is enhanced by using chilled Buffer N3 and incubating on ice. After addition of Buffer N3, a fluffy material forms and the lysate becomes less viscous. The precipitated material contains genomic DNA, proteins, cell debris, and SDS.



KiaSpin® Plasmid Purification procedure

Standard Protocol:

This protocol is designed for preparation of up to 10-40 μg of high-copy plasmid DNA from 1-5 ml overnight cultures of E.coli in LB medium.
Please take the time to read this protocol. An experienced user protocol is included for users already familiar with the procedure.

Important points before starting

• New users are strongly advised to read manual craefully .
• If working with low-copy vectors, it may be beneficial to increase the lysis buffer volumes in order to increase the efficiency of alkaline lysis, and thereby the DNA yield. In case additional Buffers P1, P2, and N3 are needed, the buffers may be purchased separately.

Important Notes for KiaSpin® Procedures

Please read the following notes before starting any of the KiaSpin® procedures.

Things to do before starting

- Add the provided RNase A solution to Buffer P1 before use. Use one vial of RNase A (spin down briefly before use) per bottle of Buffer P1, to give a final concentration of 100 μg/ml and store at 2-8°c.
- Check Buffers P2 and N3 before use for salt precipitation. Redissolve any precipitate by warming to 37 °C. Do not shake Buffer P2 vigorously.
- Pre-chill Buffer N3 to 4°C.
- Close the bottle containing Buffer P2 immediately after use to avoid acidification of Buffer P2 from CO_2 in the air.
- Buffers N3 contain irritants. Wear gloves when handling these buffers.

Host strains

Most E. coli strains can be used successfully to isolate plasmid DNA, although the strain used to propagate a plasmid can have a substantial influence on the quality of the purified DNA. Host strains such as DH1, DH5α™, and C600 yield high-quality DNA with KIAGEN protocols. The slower growing strain XL1-Blue also yields DNA of very high quality which works extremely well for sequencing. Strain HB101 and its derivatives, such as TG1 and the JM100 series, contain large amounts of carbohydrates that are released during lysis and can inhibit enzyme activities if not completely removed (3). In addition, some strains such as JM101, JM110, and HB101 have high levels of endonuclease activity, and yield DNA of lower quality than that prepared from strains such as XL1-Blue, DH1, DH5α, and C600. The methylation and growth characteristics of the host strain can also affect plasmid isolation. If after performing a KIAGEN plasmid preparation, the quality of purified DNA is not as expected, a change of host strain should be considered. If difficulty is encountered with strains such as TG1 and Top10F', we recommend either reducing the amount of culture volume used for cleared lysate preparation, or using the same amount of culture volume but doubling the volumes of Buffers P1, P2, and N3 in order to improve the ratio of biomass to lysis buffers for optimized lysis conditions.

Inoculation

Bacterial cultures for plasmid preparation should always be grown from a single colony picked from a freshly streaked selective plate. Subculturing directly from glycerol stocks, agar stabs, and liquid cultures is poor microbiological practice and may lead to loss of the plasmid. Inoculation from plates that have been stored for a long time may also lead to loss or mutation of the plasmid.
The desired clone should be streaked from a glycerol stock onto a freshly prepared agar plate containing the appropriate selective agent such that single colonies can be isolated. This procedure should then be repeated to ensure that a single colony of an antibiotic-resistant clone can be picked. A single colony should be inoculated into 2–10 ml of LB medium containing the appropriate selective agent and grown for ~8 hours (logarithmic phase). Using a vessel with a volume of at least four times

greater than the volume of medium, the starter culture should then be diluted 1/500 to 1/1000 into a larger volume of selective medium, and grown with vigorous shaking (~300 rpm) to saturation (12–16 hours). It is often convenient to grow the starter culture during the day and the larger culture overnight for harvesting the following morning.

Antibiotics

Antibiotic selection should be applied at all stages of growth. Many plasmids in use today do not contain the par locus which ensures that the plasmids segregate equally during cell division in the absence of selective pressure. Daughter cells that do not receive plasmids will replicate much faster than plasmid-containing cells and can quickly take over the culture. The stability of the selective agent should also be taken into account. Resistance to ampicillin, for example, is mediated by β-lactamase which is encoded by the plasmid linked bla gene and which hydrolyzes ampicillin. Levels of ampicillin in the culture medium are thus continually depleted. This phenomenon is clearly demonstrated on ampicillin plates, where "satellite colonies" appear as the ampicillin is hydrolyzed in the vicinity of a growing colony. It is important to inoculate cultures from freshly prepared plates to ensure that the antibiotic is effective. Ampicillin is also very sensitive to temperature, and should be stored frozen in single-use aliquots. Table 2 gives the concentrations of commonly used antibiotics.

Table 2. Concentrations of commonly used antibiotics

Antibiotic	Stock solutions		Working concentration
	Concentration	Storage	(dilution)
Ampicillin (sodium salt)	50 mg/ml in water	–20°C	100 µg/ml (1/500)
Chloramphenicol	34 mg/ml in ethanol	–20°C	170 µg/ml (1/200)
Kanamycin	10 mg/ml in water	–20°C	50 µg/ml (1/200)
Streptomycin	10 mg/ml in water	–20°C	50 µg/ml (1/200)
Tetracycline HCl	5 mg/ml in ethanol	–20°C	50 µg/ml (1/100)

KIAGEN protocols are optimized for use with cultures grown in standard Luria Bertani (LB) medium, grown to a cell density of approximately 3-4 x 10^9 cells per ml. We advise harvesting cultures after approximately 12–16 hours of growth, which typically is the transition from logarithmic into stationary growth phase. At this time, the ratio of plasmid DNA to RNA is higher than during the logarithmic phase. Also, the DNA is not yet degraded due to overaging of the culture, as in the later stationary phase.

Luria-Bertani (LB) broth is the recommended culture medium for use with KiaSpin® Plasmid Extract Kits, since richer broths such as TB (Terrific Broth) or 2x YT lead to extremely high cell densities which can overload the purification system. It should be noted that cultures grown in TB may yield 2-5 times the number of cells compared to cultures grown in LB. If these media are used, recommended culture volumes must be reduced to match the capacity of the **KiaSpin® membrane**. If too much culture volume is used, alkaline lysis will be inefficient, the **KiaSpin® membrane** will be overloaded, and the performance of the system will be unsatisfactory. Furthermore, the excessive viscosity of the lysate will require vigorous mixing, which may result in shearing of bacterial genomic DNA and contamination of the plasmid DNA. Care must also be taken if strains are used which grow unusually fast or to very high cell densities. It is best to calculate culture cell density and adjust the volume accordingly.

Please note that a number of slightly different LB culture broths, containing different concentrations of NaCl, are in common use. Although different LB broths produce similar cell densities after overnight culture, plasmid yields can vary significantly. We recommend growing cultures in LB medium containing 10g NaCl, 10g tryptone, and 5g yeast extract per liter to obtain the highest plasmid yields.

Contents	LB-Miller	LB-Lennox
Tryptone	10 g	10 g
Yeast extract	5 g	5 g
NaCl	10 g	5 g