

Troubleshooting Tips

Problem	Likely cause	Suggestion
Low DNA yields.	Poor cell lysis.	<ul style="list-style-type: none"> • Only use LB or YT medium containing ampicillin. • Do not use more than 5 ml (HC plasmids) or 10 ml (LC plasmids) culture with the basic protocol. • Cells may not be dispersed adequately prior to addition of Solution II. Vortex cell suspension to completely disperse. • Increase incubation time with Solution II to obtain a clear lysate. • Solution II, if not tightly closed, may need to be replaced. Prepare as follows: 0.2 N NaOH, 1% SDS.
	Bacterial culture overgrown or not fresh.	<ul style="list-style-type: none"> • Do not incubate cultures for more than 16 hr at 37°C and don't use stored cultures.
	Low copy-number plasmid used.	<ul style="list-style-type: none"> • Such plasmids may yield as little as 0.5µg DNA from a 5 ml overnight culture. Increase overnight culture volume to 10 ml.
No DNA eluted.	Wash Buffer Concentrate not diluted with absolute ethanol.	<ul style="list-style-type: none"> • Dilute Wash Buffer with 100% ethanol as instructed above.
High molecular weight DNA contamination of product.	Over mixing of cell lysate upon addition of Solution II.	<ul style="list-style-type: none"> • Do not vortex or mix aggressively after adding Solution II. Adequate mixing is obtained by simply inverting and rotating tube to cover walls with viscous lysate.
Optical densities do not agree with DNA yield on agarose gel.	Trace contaminants eluted from column increase A 260.	<ul style="list-style-type: none"> • Make sure to wash column as instructed in steps 5 and 7. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantitation.
RNA visible on agarose gel.	RNase A not added to Solution I.	<ul style="list-style-type: none"> • Add RNase A to Solution I before use.
Plasmid DNA floats out of well while loading agarose gel.	Ethanol not completely removed from column following wash steps.	<ul style="list-style-type: none"> • Centrifuge column as instructed in step 8 to dry completely.

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Introduction

The E.Z.N.A.[®] family of products is an innovative system that radically simplifies extraction and purification of nucleic acids from a variety of sources. Key to the system is the HiBind[®] matrix that avidly, but reversibly, binds DNA or RNA under certain optimal conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or low salt buffer.

The Plasmid Miniprep Kit combines the power of HiBind[®] technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high quality DNA. HiBind[®] mini-columns facilitate the binding, washing, and elution steps thus enabling multiple samples to be simultaneously processed. Yields vary according to plasmid copy number, *E.coli* strain and conditions of growth, but 5 ml of overnight culture with high copy-number plasmids in LB medium typically produces 15-25 µg plasmid DNA. For more DNA, we recommend the Plasmid Miniprep Kit II that yields 40-75 µg DNA from 10-15 ml culture when using high copy-number plasmids.

Plasmid-DNA isolated with the E.Z.N.A.[®] Plasmid Miniprep Kit is suitable for automated fluorescent DNA sequencing, restriction endonuclease digestion, and other applications.

E.Z.N.A.[®] Plasmid Mini Kits I are available as S- or C-line columns (Safety-Line, Best.-Nr. 12-6943-xx or Classic Line, Best.-Nr. 12-6943-xx). S-line columns have a slender form and a tube-like outlet at the bottom, ensuring that all positions in centrifuges and vacuum manifolds can be occupied. Lids close the columns tightly and avoid cross-contamination. C-line columns have a bigger diameter and a flat bottom, allowing higher sample volumes to be processed. These columns possess no lid.

Alternatively, quantity (as well as quality) can sometimes best be determined by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations.

Storage of DNA

DNA purified by E.Z.N.A.[®] Mini Prep Kits can be stored in TE Buffer or sterile, deionized water at -20 °C for years.

Ordering information

For plasmid isolation:

E.Z.N.A. [®] Plasmid Miniprep Kit I (Up to 25 µg DNA)	12-6942-00	5 Preparations
(C-Line)	12-6942-01	50 Preparations
	12-6942-02	200 Preparations
E.Z.N.A. [®] Plasmid Miniprep Kit I (Up to 25 µg DNA)	12-6943-00	5 Preparations
(S-Line)	12-6943-01	50 Preparations
	12-6943-02	200 Preparations
E.Z.N.A. [®] Plasmid Miniprep Kit II (Up to 75 µg DNA)	12-6945-00	5 Preparations
(C-Line)	12-6945-01	50 Preparations
	12-6945-02	200 Preparations
E.Z.N.A. [®] Plasmid Miniprep Kit II (Up to 75 µg DNA)	12-6946-00	5 Preparations
(S-Line)	12-6946-01	50 Preparations
	12-6946-02	200 Preparations
E.Z.N.A. [®] Plasmid Maxi Kit I (Up to 1.5 mg DNA)	12-6922-01	5 Preparations
	12-6922-02	20 Preparations
E.Z.N.A. [®] HP Plasmid Mini Kit I (Up to 25 µg DNA)	12-7042-01	50 Preparations
	12-7042-02	200 Preparations
E.Z.N.A. [®] HP Plasmid Mini Kit II (Up to 75 µg DNA)	12-7045-01	50 Preparations
	12-7045-02	200 Preparations

7. Wash III (optional)

Repeat wash step with another 750 µl Wash Buffer, as described in step 6.

8. Dry (Important, do not skip this step!)

Place the HiBind® miniprep column containing your plasmids in the collection tube used in step 7 and centrifuge for 1 min at 10'000 x g to dry the column matrix. This step is essential to remove ethanol from the column.

9. Elution

Place the HiBind® miniprep column (step 8) into a fresh 1.5 ml microcentrifuge tube. Add 50 - 100 µl (depending on the desired concentration of final product) sterile deionized water or TE buffer directly to the binding matrix in the HiBind® miniprep column and centrifuge for 1 min at 10'000 x g to elute DNA.

The first elution represents approximately 75-80% of the bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

Discard the HiBind® miniprep column and store the eluted plasmid DNA at +4 °C or at -20 °C.

Yield and quality of DNA

Determine the absorption of an appropriate dilution (20- to 50-fold) of the sample at 260 nm and then at 280 nm. One A_{260} -unit is about 50µg DNA/ml. The DNA concentration is calculated as follows:

$$\text{DNA conc. } (\mu\text{g/ml}) = \text{Absorption}_{260} \times 50 \times \text{Dilution Factor}$$

High copy number plasmids generally yield up to 25 µg of DNA from 5 ml culture. The ratio of $A_{260/280}$ is an indication of nucleic acid purity. A value higher than 1.8 indicates > 90% nucleic acid. Typically, the majority of the DNA eluted is in monomeric supercoiled form, though concatamers may also be present.

Kit Components

E.Z.N.A.® Plasmid Miniprep Kit I	5 Purifications	50 Purifications	200 Purifications
Product Number	12-6942-00	12-6942-01	12-6942-02
	12-6943-00	12-6943-01	12-6943-02
Components			
HiBind® Columns	5	50	200
2 ml Collection Tubes	5	50	200
Solution I	5 ml	20 ml	60 ml
Solution II	5 ml	20 ml	60 ml
Solution III	5 ml	20 ml	2 x 40 ml
Buffer HB	5 ml	25 ml	100 ml
DNA Wash buffer	12 ml	40 ml	3 x 40 ml
RNase A	50 µl	100 µl	400 µl
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Storage and Stability

All E.Z.N.A.® Plasmid Miniprep Kit I components are stable for at least 12 months from the date of purchase when stored as follows: Solution I/RNase A at 4 °C, all other components at room temperature (22 °C – 25 °C).

Before Starting

Briefly examine this booklet and become familiar with each step. Prepare all components and have the necessary materials ready before starting.

- ! Before using, Solution I must be completed with RNase A. Add the volume of delivered RNase A to the bottle of Solution I provided and mix carefully. Store the completed Solution I at 4 °C.
- ! Solution II should be closed firmly when not used.
- ! DNA Wash Buffer is concentrated and has to be diluted with absolute ethanol as follows:

Kit 12-6942-00	Add 18 ml 100% EtOH to 12 ml wash buffer
Kit 12-6942-01	Add 60 ml 100% EtOH to 40 ml wash buffer
Kit 12-6942-02	Add 3 x 60 ml 100% EtOH to 3 x 40 ml wash buffer

Store diluted DNA Wash Buffer at room temperature.

- ! All steps must be carried out at room temperature.

3. Neutralisation of Lysate

Add 350 µl (for 5 ml overnight culture) or 700 µl (for 10 ml overnight culture) Solution III to the cleared lysate and gently mix by inverting the tube several times until a flocculent white precipitate forms. Centrifuge at 10'000 x g for 10 minutes at room temperature.

4. Load and bind

Transfer the clear supernatant (step 3) to a fresh HiBind® miniprep column in a 2 ml collection tube. Ensure that the pellet is not disturbed and that no cellular debris are carried over into the column. Centrifuge the column / collection tube assembly for 1 min at 10'000 x g at room temperature to completely pass lysate through the membrane.

Discard the flow-through liquid and keep the collection tube for further steps.

5. Wash I (optional)

Place the HiBind® miniprep column (step 4) in the collection tube used in step 4 and add 500 µl HB Buffer. Centrifuge for 1 min at 10'000 x g. Discard the flow-through liquid and keep the collection tube for further steps.

This wash step with HB buffer leads to an efficient removal of protein contamination and is recommended for sensitive further experiments. For standard applications, like cloning screening, you may skip this step.

6. Wash II

Place the HiBind® miniprep column (step 5) in the collection tube used in step 5 and add 750 µl of Wash Buffer diluted with ethanol. Centrifuge for 1 min at 10'000 x g, discard the flow-through and keep the collection tube for further steps.

Delivered Wash Buffer must be diluted with absolute ethanol before use. If refrigerated, Wash Buffer must be brought to room temperature.

B. Low Copy-Number Plasmids

1. Bacterial Culture

Inoculate 10 ml growth medium containing the appropriate antibiotic in a 20-50 ml culture tube with *E.coli* carrying the desired plasmid and grow at 37°C with shaking for 12-16 h.

It is strongly recommended to use an endA⁻ strain of E.coli for routine plasmid isolation. Examples of such strains include DH5 α and JM109. For endA⁺ E. coli strains we recommend the E.Z.N.A.[®] HP Plasmid Prep Kit.

Pellet 5 or 10 ml overnight culture by centrifugation for 1 min at 10'000 x g in several 1.5 ml centrifuge tubes or for 10 min at 5'000 x g in one 15 ml centrifuge tube. Pour off supernatant and discard.

For Low-Copy Number Plasmids, do not use more than 10 ml overnight culture, since the HiBind[®] mini-column quickly becomes saturated. In this situation we recommend processing of multiple samples from the same culture. Alternatively, work with the E.Z.N.A.[®] Plasmid Miniprep Kit II (product No. 12-6945), that allows processing of 25-100 ml cultures using the mini-column format and generally yields 20-70 μ g plasmid DNA.

2. Lysis of bacteria

Add 250 μ l (for 5 ml overnight culture) or 500 μ l (for 10 ml overnight culture) Solution I/RNase A to the bacterial pellet, put the lid back on the tube and completely resuspend cells by vortexing.

Complete resuspension of cell pellet is critical for obtaining high yields.

Add 250 μ l (for 5 ml overnight culture) or 500 μ l (for 10 ml overnight culture) Solution II and gently mix by inverting and rotating tube 4- 6 times to obtain a cleared lysate. A 2-min incubation at room temperature may be necessary.

Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Store Solution II tightly capped when not in use.

E.Z.N.A.[®] Plasmid Miniprep Kit I Protocol

Materials required, but not supplied:

- ! Appropriate Bacterial Growth Medium
- ! 100 % Ethanol
- ! TE buffer or sterile deionized water
- ! Sterile pipet tips and centrifuge tubes

A. High Copy-Number Plasmids

1. Bacterial Culture

Inoculate 5 ml growth medium containing the appropriate antibiotic in a 10-20 ml culture tube with *E.coli* carrying the desired plasmid and grow at 37°C with shaking for 12-16 h.

It is strongly recommended to use an endA⁻ strain of E.coli for routine plasmid isolation. Examples of such strains include DH5 α and JM109. For endA⁺ E. coli strains we recommend the E.Z.N.A.[®] HP Plasmid Prep Kit.

Pellet overnight culture by centrifugation for 1 min at 10'000 x g in 1.5 ml centrifuge tubes or for 10 min at 5'000 x g in a 15 ml centrifuge tube. Pour off supernatant and discard.

For High-Copy Number Plasmids, do not use more than 5 ml overnight culture, since the HiBind[®] mini-column quickly becomes saturated. In this situation we recommend processing of multiple samples from the same culture. Alternatively, work with the E.Z.N.A.[®] Plasmid Miniprep Kit II (product No. 12-6945), that allows processing of 10-15 ml culture using the mini-column format and generally yields 40-75 μ g plasmid DNA.

2. Lysis of bacteria

Add 250 μ l Solution I/RNase A to the bacterial pellet, put the lid back on the tube and completely resuspend cells by vortexing.

Complete resuspension of cell pellet is critical to obtaining high yields.

Add 250 µl Solution II and gently mix by inverting and rotating tube 4- 6 times to obtain a cleared lysate. A 2-min incubation at room temperature may be necessary.

Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Store Solution II tightly capped when not in use.

3. Neutralisation of Lysate

Add 350 µl Solution III to the cleared lysate and gently mix by inverting the tube several times until a flocculent white precipitate forms. Centrifuge at 10'000 x g for 10 minutes at room temperature.

4. Load and bind

Transfer the clear supernatant (step 3) to a fresh HiBind® miniprep column in a 2 ml collection tube. Ensure that the pellet is not disturbed and that no cellular debris are carried over into the column. Centrifuge the column / collection tube assembly for 1 min at 10'000 x g at room temperature to completely pass lysate through the membrane.

Discard the flow-through liquid and keep the collection tube for further steps.

5. Wash I (optional)

Place the HiBind® miniprep column (step 4) in the collection tube used in step 4 and add 500 µl HB Buffer. Centrifuge for 1 min at 10'000 x g. Discard flow-through liquid and keep the collection tube for further steps.

This wash step with HB buffer leads to an efficient removal of protein contamination and is recommended for further sensitive experiments. For standard applications, like cloning screening, you may skip this step.

6. Wash II

Place the HiBind® miniprep column (step 5) in the collection tube used in step 5 and add 750 µl of Wash Buffer diluted with ethanol.

Centrifuge for 1 min at 10'000 x g, discard the flow-through and keep the collection tube for further steps.

Delivered Wash Buffer must be diluted with absolute ethanol before use. If refrigerated, Wash Buffer must be brought to room temperature before use.

7. Wash III (optional)

Repeat wash step with another 750 µl Wash Buffer, as described in step 6.

8. Dry (Important, do not skip this step!)

Place the HiBind® miniprep column containing your plasmids in the collection tube used in step 7 and centrifuge for 1 min at 10'000 x g to dry the column matrix. This step is essential to remove ethanol from the column.

9. Elution

Place the HiBind® miniprep column (step 8) into a fresh 1.5 ml microcentrifuge tube. Add 50 - 100 µl (depending on desired concentration of final product) sterile deionized water or TE buffer directly to the binding matrix in the miniprep column and centrifuge for 1 min at 10'000 x g to elute DNA.

The first elution represents approximately 75-80% of the bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

Discard the HiBind® miniprep column and store the eluted plasmid DNA at +4 °C or at -20 °C.

E.Z.N.A.[®] Plasmid Miniprep

Kit I

- Instruction Manual -