

# **peqGOLD**

## **HP Plasmid Miniprep Kit I**

**– Instruction Manual –**

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## Introduction

The peqGOLD 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 HP Plasmid Miniprep Kit I 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 in LB medium typically produces 15 - 25 µg plasmid DNA. For more DNA, we recommend the HP Plasmid Miniprep Kit II that yields up to 75 µg DNA from 5 ml culture when using high copy-number plasmids.

The peqGOLD High Performance (HP) plasmid purification system is the modified version of peqGOLD plasmid isolation system which is designed specially for those applications when high quality plasmid is required such as transfection, autosequencing, etc. It also suitable for isolating plasmids from bacterial hosts (such as EndA<sup>+</sup> strains) with high level of endonuclease activity. Plasmids from this system have a better stability for long-term storage.

Plasmid-DNA isolated with the peqGOLD HP Plasmid Miniprep Kit I is suitable for automated fluorescent DNA sequencing, restriction enzyme digestion, and other applications.

## Kit Components

peqGOLD HP Plasmid Miniprep Kit I	50 Purifications	200 Purifications
Product Number	12-7042-01	12-7042-02
<b>Components</b>		
HiBind® Columns	50	200
2 ml Collection Tubes	50	200
Solution I	30 ml	120 ml
Solution II	30 ml	120 ml
Solution III	40 ml	2 x 80 ml
Buffer HB	30 ml	110 ml
DNA Wash buffer	40 ml	3 x 40 ml
RNase A	100 µl	400 µl
OB™ Protease Mix	15 mg	50 mg
10 mM TE Buffer	1.5 ml	2 x 1.5 ml
Elution buffer	15 ml	60 ml

## Storage and Stability

All peqGOLD HP Plasmid Miniprep Kit I components are stable for at least 12 months from the date of purchase when stored as follows: RNase A, Solution I / RNase A at 4 °C, dissolved OB protease mixture at -20 °C, all other components at room temperature (22 -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 12-7042-01	Add 60 ml 100% EtOH to 40 ml wash buffer.
Kit 12-7042-02	Add 3 x 60 ml 100% EtOH to 3 x 40 ml wash buffer.

Store diluted DNA Wash Buffer at room temperature.

- ! The OB Protease mixture is delivered as powder and has to be dissolved in TE Buffer or sterile, deionized water before use:

Kit 12-7042-01	Dissolve 15 mg Protease mixture in 600 µl TE Buffer.
Kit 12-7042-02	Dissolve 50 mg Protease mixture in 2 ml TE Buffer

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

## peqGOLD HP Plasmid Miniprep Kit I Protocol

Materials required, but not supplied:

- ! Appropriate Bacterial Growth Medium
- ! 100 % Ethanol
- ! Sterile deionized water (optional)
- ! 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.

*For routine plasmid isolations using endA<sup>-</sup> strains of E.coli, like DH5 $\alpha$  and JM109, you may use the standard peqGOLD Plasmid Miniprep Kits.*

Pellet overnight culture by centrifugation for 1 min at 10,000 x g in a 1,5 ml centrifuge tube or 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<sup>®</sup> mini-column quickly becomes saturated. Processing of 5 ml culture using the HP Mini Prep Kit I generally yields 15-25  $\mu$ g plasmid DNA. For more DNA we recommend processing of multiple samples from the same culture or using the HP Mini Prep Kit II.*

#### 2. Lysis of bacteria

Add 250  $\mu$ 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 one after the other 500  $\mu$ l Solution II and 10  $\mu$ l OB protease mixture. Gently mix by inverting and rotating tube 4- 6 times to obtain a cleared lysate. Incubate at room temperature for 10 minutes.

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

### 3. Neutralization 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 is 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 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 helpful for sensitive further experiments.*

### 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 washing 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 final concentration of plasmids) Elution buffer or sterile deionized water 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.

## B. Low Copy-Number Plasmids

### 1. Bacterial Culture

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

*For routine plasmid isolations using endA<sup>-</sup> strains of E.coli, like DH5α and JM109, you may use the standard peqGOLD Plasmid Miniprep Kits.*

Pellet overnight culture by centrifugation for 10 min at 5,000 x g in a 15 ml centrifuge tube or for 1 min at 10,000 x g in several 1,5 or 2 ml centrifuge tubes. Pour off supernatant and discard.

*For Low-Copy Number Plasmids, do not use more than 100 ml overnight culture, since the HiBind mini-column quickly becomes saturated. Processing of 100 ml Low Copy-number plasmids culture using the HP Mini Prep Kit II generally yields 20-70 µg plasmid DNA. For less DNA you may use the peqGOLD HP Mini Prep Kit I to get up to 8 µg DNA from 5 to 10 ml overnight culture.*

### 2. Lysis of Bacteria

Add 250 µl (for 5 ml overnight culture) or 500 µl (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 to obtaining high yields. Depending on the bacterial strain, the volume of Solution I/RNase A may be increased for good yields up to 1250 µl / 25 ml overnight culture.*

Add 250 µl (for 5ml overnight culture) or 500 µl (for 10 ml overnight culture) Solution II and 10 µl or 20 µl OB protease mixture, respectively, to the tube and gently mix by inverting and rotating tube 4- 6 times to obtain a cleared lysate. Incubate for 10 minutes at room temperature.

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

### 3. Neutralization of Lysate

Add 350  $\mu$ l (for 5 ml overnight culture) or 700  $\mu$ l (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<sup>®</sup> miniprep column in a 2 ml collection tube. Ensure that the pellet is not disturbed and that no cellular debris is 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 and keep the collection tube for further steps.

### 5. Wash I (optional)

Place the HiBind<sup>®</sup> miniprep column (step 4) in the collection tube used in step 4 and add 500  $\mu$ l 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 helpful for sensitive further experiments.*

### 6. Wash II

Place the HiBind<sup>®</sup> miniprep column (step 5) in the collection tube used in step 5 and add 750  $\mu$ l 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.*

### 7. Wash III (optional)

Repeat wash step with another 750  $\mu$ l Wash Buffer, as described in step 6.

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

Place the HiBind<sup>®</sup> 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 final concentration of plasmids) Elution buffer or sterile deionized water 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 50  $\mu\text{g}$  DNA/ml. The DNA concentration is calculated as follows:

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

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.

*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 peqGOLD HP Miniprep Kit I can be stored in TE Buffer or sterile, deionized water at  $-20\text{ }^{\circ}\text{C}$  for years.

## Ordering information

For plasmid isolation:

peqGOLD HP Plasmid Mini Kit I <i>(Up to 25 µg DNA)</i>	12-7042-01	50 Preparations
	12-7042-02	200 Preparations
peqGOLD HP Plasmid Mini Kit II <i>(Up to 75 µg DNA)</i>	12-7045-01	50 Preparations
	12-7045-02	200 Preparations
peqGOLD HP Plasmid Midi Kit <i>(Up to 0.4 mg DNA)</i>	12-7004-01	10 Preparations
	12-7004-02	50 Preparations

## Troubleshooting Tips

Problem	Likely cause	Suggestion
Low DNA yields.	Poor cell lysis.	<ul style="list-style-type: none"> <li>• Only use LB or YT medium containing ampicillin.</li> <li>• Do not use more than 5 ml (HC plasmids) or 10 ml (LC plasmids) culture with the basic protocol.</li> <li>• Cells may not be dispersed adequately prior to addition of Solution II. Vortex cell suspension to completely disperse.</li> <li>• Increase incubation time with Solution II to obtain a clear lysate.</li> <li>• Solution II, if not tightly closed, may need to be replaced. Prepare as follows: 0.2 N NaOH, 1% SDS.</li> </ul>
	Bacterial culture overgrown or not fresh.	<ul style="list-style-type: none"> <li>• Do not incubate cultures for more than 16 hr at 37°C and don't use stored cultures.</li> </ul>
No DNA eluted.	Wash Buffer Concentrate not diluted with absolute ethanol.	<ul style="list-style-type: none"> <li>• Dilute Wash Buffer with 100% ethanol as instructed above.</li> </ul>
High molecular weight DNA contamination of product.	Over mixing of cell lysate upon addition of Solution II.	<ul style="list-style-type: none"> <li>• 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.</li> </ul>
Optical densities do not agree with DNA yield on agarose gel.	Trace contaminants eluted from column increase Absorption.	<ul style="list-style-type: none"> <li>• Make sure to wash column as instructed in step 5 and 7. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantization.</li> </ul>
RNA visible on agarose gel.	RNase A not added to Solution I.	<ul style="list-style-type: none"> <li>• Add RNase A to Solution I before use.</li> </ul>
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"> <li>• Centrifuge column as instructed in step 8 to dry completely.</li> </ul>