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Introduction

The E.Z.N.A.™ Endo-Free Plasmid Kits combine the power of HiBind® technology with the Omega Bio-Tek's innovative ETR technology to deliver high quality plasmid with low in endotoxins for use in eukaryotic transfection, and in vitro experiments.

Endotoxins are lipopolysaccharides (LPS), found in the outer cell membrane of gram negative bacteria such as E.coli. One E.coli cell contains around 2 million LPS molecules, each having hydrophobic, hydrophilic, and charged regions. Bacteria release small quantities of endotoxins during growth, and large quantities at death. At the time of lysis during plasmid purification, endotoxins are shed into the lysate. The chemical and physical properties that endotoxin molecules possess lead to their copurification with plasmid DNA by behaving similarly on the surface of silica and anion-exchange resins. The E.Z.N.A.™ Endo-Free Plasmid System uses a specially formulated buffer that prevents endotoxin molecules from binding to the surface of the HiBind® matrix. In addition the E.Z.N.A.™ Endo-Free Plasmid Midi and Maxi Kits include specialized filter cartridges that replace the centrifugation step following alkaline lysis.

For the best interpretation of results it is crucial the purified plasmid DNA be free of endotoxins. Endotoxin contamination lowers transfection efficiencies for endotoxin sensitive cell lines. For gene therapy, endotoxin contamination should be of major concern since endotoxins have the potential to cause fever, endotoxic shock syndrome, and interfere with in vitro transfection into immune cells.

New In this Edition

- A new Equilibration Buffer is introduced to Fastfilter Plasmid kits to improve the DNA yield and consistency of performance.
- New developed ETR Binding Buffer and ETR Wash Buffer effectively remove the endotoxin and simplify the procedure.

Storage and Stability

All of the E.Z.N.A.™ Fastfilter® Plasmid Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows: Solution I (once RNase A is added) at 4°C, all other material at 22-25°C. Store Solution II tightly capped when not in use.

Kit Contents

| Endo-Free Plasmid Mini Kit I | D6948-00 | D6948-01 | D6948-02 |
|-------------------------------|-----------|----------|------------|
| HiBind® DNA Mini Columns (I) | 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 |
| Buffer N3 | 2.5 ml | 10 ml | 30 ml |
| Equilibration Buffer | 1.5 ml | 7 ml | 25 ml |
| ETR Binding Buffer | 5 ml | 40 ml | 160 ml |
| ETR Wash Buffer | 5 mL | 30 mL | 120 mL |
| Buffer HB | 5 ml | 30 ml | 120 ml |
| DNA Wash Buffer | 1.5 ml | 15 ml | 3 x 25 ml |
| RNase A | Pre-added | 100 µl | 300 µl |
| Endotoxin-Free Elution Buffer | 1.2 ml | 10 ml | 30 ml |
| Instruction Booklet | 1 | 1 | 1 |
| Endo-Free Plasmid Mini Kit II | D6950-00 | D6950-01 | D6950-02 |
| HiBind® DNA Mini Columns (II) | 5 | 50 | 200 |
| 2 ml Collection Tubes | 5 | 50 | 200 |
| Solution I | 5 ml | 30 ml | 120 ml |
| Solution II | 5 ml | 30 ml | 120 ml |
| Buffer N3 | 2.5 ml | 15 ml | 60 ml |
| Equilibration Buffer | 1.5 ml | 7 ml | 25 ml |
| ETR Binding Buffer | 10 ml | 80 ml | 2 x 160 ml |
| ETR Wash Buffer | 5 mL | 30 mL | 120 mL |
| Buffer HB | 5 ml | 30 ml | 120 ml |
| DNA Wash Buffer | 1.5 ml | 15 ml | 3 x 25 ml |
| RNase A | pre-added | 100 µl | 400 µl |
| Endotoxin-Free Elution Buffer | 1.5 ml | 15 ml | 40 ml |
| Instruction Booklet | 1 | 1 | 1 |

*Elution Buffer = 10 mM Tris-HCl (pH 8.5)

CAUTION! Buffer ETR Binding Buffer and HB contains Chaotropic salts. Equilibration Buffer contains Sodium Hydroxide. Use gloves and protective eyewear when handling this solution.

Before Starting

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

- Add vial of RNase A to the bottle of Solution I provided and store at 4°C.
- Dilute DNA Wash Buffer with absolute ethanol (96-100%) as follows:

E.Z.N.A.™ Fastfilter® Plasmid Mini Kit I

D6948-00 Add 6 ml of absolute ethanol to bottle
 D6948-01 Add 60 ml of absolute ethanol to bottle
 D6948-02 Add 100 ml of absolute ethanol to bottle

E.Z.N.A.™ Fastfilter® Plasmid Mini Kit II

D6950-00 Add 60 ml of absolute ethanol to bottle
 D6950-01 Add 60 ml of absolute ethanol to bottle
 D6950-02 Add 100 ml of absolute ethanol to bottle

Store diluted DNA Wash Buffer at room temperature !

- Check Solution II and ETR Binding Buffer for salt precipitation before use. Redissolve any precipitation by warming to 37° C.
- Store Solution II Tightly Capped when not in use.

Equipments and Reagents Supplied by User

- Absolute ethanol (96-100%)
- Centrifuge capable of 13,000 x g
- 1.5 ml Centrifuge tubes
- Vacuum Manifold (OPTIONAL for Vacuum protocol)
- ddH₂O(for vacuum protocols)

Growth and Culture of Bacteria

Bacterial Strain Selection

It is strongly recommended that an endA negative strain of E.coli be used for routine plasmid isolation. Examples of such strains include DH5 α ®, DH 1, and C600. These host strains yield high quality DNA with E.Z.N.A.™ Plasmid Isolation Protocols. XL1-Blue, although a slower growing strain is also recommended due to its yield of high quality DNA.

Host strains derivatives from HB101 such as TG1 and the JM100 series release large amounts of carbohydrates during lysis, which may inhibit enzyme activities when not completely removed. Some strains may also lower DNA quality due to having high levels of endonuclease activity, and therefore are not recommended (i.e. JM101, JM110, HB101). One may reduce the amount of culture volume or double the volumes of Solution I, II, and III, if problems are encountered with strains such as TG1 and Top10F.

Inoculation

Bacterial cultures for plasmid preparations should always be grown from a single colony picked from a freshly streaked plate. Subculturing directly from glycerol stock or liquid cultures may lead to uneven yields or plasmid loss. Optimal results are obtained by using one single isolated colony from a freshly transformed or freshly streaked plate to inoculate an appropriate volume of starter culture containing the appropriate antibiotic, and then incubated for 12-16 at 37°C with vigorous shaking (~300rpm; shaking incubator). NOTE: Aeration is very important. The culture volume should not exceed 1/4 the volume of the container.

Culture Media

The E.Z.N.A.™ Plasmid Kits are specially designed for use with cultures grown in *Luria Bertani (LB) medium*. Richer broths such as TB(Terrific Broth) or 2 x YT lead to high cell densities that can overload the purification system, and therefore are not recommended. If rich media has to be used, growth times have to be optimized, and the recommended culture volumes must be reduced to match the capacity of the HiBind® spin column.

NOTE: As culture ages DNA yield may begin to decrease due to cell death and lysis within the culture.

Culture Volume and Cell Density

DO NOT EXCEED MAXIMUM RECOMMENDED CULTURE VOLUMES

For optimal plasmid yields, the starting culture volume should be based on culture cell density. A bacterial density between 2.0 and 3.0 at OD600 is recommended. When using nutrient-rich media, care should be taken to ensure that the cell density does not exceed an OD600 of 3.0. Using a high-density culture outside of the recommended OD range may overload the purification system.

Low Copy-Number Plasmid and Cosmid DNA Purification

Low copy number plasmids generally give 0.1-1 μ g DNA per ml overnight culture. For the isolation of plasmid DNA from low copy-number plasmids (0.1-1 μ g/ml culture) or low midi copy-number plasmid (1-2 μ g/ml culture) bacteria, use the following modified protocol.

1. Starting bacterial volume: Double the volume of starting culture from that of high copy number plasmids. Use up to 10 ml for Mini kit I and 20-30 ml for Mini Kit II. Pellet the bacterial cells by centrifugation.
2. Perform resuspension, lysis and N3 steps by using double volumes of Solution I, Solution II, Buffer N3 and ETR Binding Buffer. *Additional buffer for Solution I, Solution II, Buffer N3 and ETR Binding can be purchased separately.*
3. Continue with each step of the standard protocol by following the wash, drying and elution steps. There is no need to increase the volumes of Buffer HB, ETR Wash Buffer, DNA Wash Buffer and Elution Buffer.

Endo-Free Plasmid Mini Kit I Spin Protocol

For the isolation of 8-40µg of high-copy plasmid DNA from a 1-5ml culture using the E.Z.N.A.™ spin-column format.

For low-copy-number plasmids, larger volumes (5-10ml) of bacterial culture must be processed, and buffer volumes will double (to ensure optimal DNA recovery).

Proceed to the "Low-copy-number plasmids Protocol" (yields = 1-10µg)

Things to do before starting:

Pre-Chill Buffer N3 on ice

Preheat Elution Buffer to 70°C if Plasmid DNA is >10kb

Prepare DNA Wash Buffer and Solution 1 according to directions on page 4

1. Obtain a single isolated colony from a fresh LB/ampicillin (50 µg/ml) agar plate and inoculate a culture of 1-5 ml LB/ampicillin medium placed in a 10-20 ml culture tube with E.coli carrying desired plasmid and grow at 37°C with agitation for 12-16 h. It is strongly recommended that an endA negative strain of E.coli be used for routine plasmid isolation.

NOTE: An OD600 reading between 2.0 and 3.0 is an indication that bacterial cells are at an optimal density for Harvesting, and plasmid DNA isolation.

IMPORTANT: DO NOT EXCEED MAXIMUM CULTURE VOLUMES!

High-copy-number maximum culture volume = 5 ml

2. Harvest the bacterial cells by centrifugation at 10,000 x g for 1 min at room temperature.
3. Decant or aspirate medium. To ensure that all traces of the medium are removed, use a clean paper towel to blot excess liquid from the wall of the vessel.
4. Add 250 µl Solution I/RNase A to the bacterial pellet. Resuspend cells completely by vortexing or pipetting. Transfer the resuspended cells to a sterile 1.5 ml microcentrifuge tube.

NOTE: Complete resuspension of cell pellet is vital for obtaining good yields.

DO NOT VORTEX FOLLOWING STEP 4 , DOING SO WILL SHEAR CHROMOSOMAL DNA.

5. Add 250 µl Solution II and mix GENTLY but thoroughly by inverting and rotating the tube 4-6 times to obtain a cleared lysate. A 1-2 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.)
 6. Add 125 µl ice-cold Buffer N3 and mix GENTLY but thoroughly by inverting the tube several times until a flocculent white precipitate forms. Centrifuge at maxi speed ($\geq 13,000 \times g$) for 10 minutes at room temperature
 7. Add Equal volume of ETR Binding Buffer to the cleared lysate. Mix thoroughly by inverting the tube 10 times.
 8. Take a HiBind® DNA Mini Column pre-inserted in a 2ml collection tube. Add 100 µl Equilibration Buffer into the column. Centrifuge at 12,000 x g for 30 seconds.
 9. Add 700 µl of the cleared lysate from step 7 into the Mini column and centrifuge at 13,000 x g for 1 minutes. Discard the flow-through and re-use the collection tube.
 10. Repeat step 9 until all of the cleared lysate pass through the Mini column.
 11. Add 500 µl of ETR Wash Buffer to the Mini column and centrifuge at 13,000 x for 30 seconds. Discard the flow-through and re-use the collection tube.
 12. Add 500 µl of Buffer HB to the Midi column and centrifuge at 13,000 x g for 30 seconds. Discard the flow-through and re-use the collection tube.
 13. Add 700 µl DNA Wash Buffer diluted with absolute ethanol to the Mini column and centrifuge at 13,000 x g for 30 seconds. Discard the flow-through and re-use the collection tube.
- Note: DNA Wash Buffer must be diluted with absolute ethanol before use. See label for directions. If refrigerated, DNA Wash Buffer must be brought to the room temperature before use.
14. Add another 700 µl DNA Wash Buffer diluted with absolute to the Mini column and centrifuge at 13,000 x g for 30 seconds. Discard the flow-through and re-use the collection tube.
 15. Centrifuge the empty column at 13,000 x g for 1 minute to dry the column matrix. Do not skip this step - it is critical for removing ethanol from the column.
 16. Place column into a clean 1.5 ml centrifuge tube. Add 50-100 µl (depending on desired final concentration)of Endo-Free Elution Buffer (10 mM Tris, pH8.5) onto the column matrix and let it sit at room temperature for 2-3 minutes. Centrifuge at 13,000 x g) for 1 min to elute DNA. This represents approximately 60-80% of bound DNA. An optional second elution will yield any residual DNA,

though at a lower concentration. Alternatively, a second elution may be performed using the first eluate to maintain a high DNA concentration. Preheating the water to 65°C and allowing the column to soak 2 min at room temperature before elution may significantly increase yields.

Endo-Free Plasmid Mini Kit I Vacuum/Spin Protocol

Things to do before starting:

Pre-Chill Buffer N3 on ice

Preheat Elution Buffer to 70°C if Plasmid DNA is >10kb

Prepare DNA Wash Buffer and Solution1 according to directions on page 4

1. Prepare cell lysate by following step 1-7 from the spin protocol on page 8-9.
2. Prepare the vacuum manifold by following the manufacturer's instructions. Insert the HiBind® Mini DNA Column into a luer connector on the manifold.
3. Equilibrate the column: Add 100 µl Equilibration Buffer into the column, wait 3 minutes. Turn on the vacuum to draw the liquid through the membrane. Add 100 µl water to the column and continue the vacuum until all the liquid pass through the column. Turn off the vacuum.
4. Pour the lysate from step 7 to the Mini column. Switch on the vacuum source to draw the solution through the column, keep adding the lysate until all the sample passes through the column and immediately switch off the vacuum source when lysate passes through the membrane .

IMPORTANT: It is important to keep the membrane wet for next wash step. If the membrane is dried, the flow rate will be significant reduced due to foaming of the detergent in the ETR Binding Buffer.

5. Add 500 µl ETR Wash Buffer to the column. Switch on the vacuum source to draw the solution through the column, and then switch off the vacuum source.
6. Apply 500 µl Buffer HB to the Mini column. Switch on the vacuum source to draw the solution through the column, and then switch off the vacuum source.
7. Apply 700 µl DNA Wash Buffer to the Mini column. Switch on the vacuum source to draw the solution through the column.
8. Apply another 700µl DNA Wash Buffer to the Mini column. Switch on

the vacuum source to draw the solution through the column. After the solution has moved through the column, continue with the vacuum for another 10 minutes to dry the membrane. Switch off the vacuum source.

9. Place the column into a 2 ml centrifuge tube(provided). Centrifuge the empty column at 12,000 x g for 2 min to dry the column matrix. Do not skip this step - it is critical for removing ethanol from the column.
10. Place column into a clean 1.5 ml centrifuge tube. Add 50-100 μ l (depending on desired final concentration)of Endo-Free Elution Buffer (10 mM Tris, pH8.5) onto the column matrix and let it sit at room temperature for 2-3 minutes. Centrifuge at 12,000 x g for 1 minute to elute DNA. An optional second elution will yield any residual DNA, though at a lower concentration. Alternatively, a second elution may be performed using the first eluate to maintain a high DNA concentration. Preheating the water to 65°C and allowing the column to soak 2 min at room temperature before elution may significantly increase yields.

Endo-Free Plasmid Mini Kit II Spin Protocol

For the isolation of 40-70 μ g of high-copy plasmid DNA from a 10-15 ml culture using the E.Z.N.A.™ spin-column format.

For low-copy-number plasmids, larger volumes (15-25ml) of bacterial culture must be processed, and buffer volumes will double (to ensure optimal DNA recovery).

Refer to the “Low-copy-number plasmids Protocol” (yields = 2-20 μ g).

Things to do before starting:

Pre-Chill Buffer N3 on ice

Preheat Elution Buffer to 70°C if Plasmid DNA is >10kb

Prepare DNA Wash Buffer and Solution 1 according to directions on page 4

1. Obtain a single isolated colony from a fresh LB/ampicillin (50 μ g/ml) agar plate and inoculate a culture of 1-15 ml LB/ampicillin medium placed in a 10-20 ml culture tube with E.coli carrying desired plasmid and grow at 37°C with agitation for 12-16 h. It is strongly recommended that an endA negative strain of E.coli be used for routine plasmid isolation.

NOTE: An OD600 reading between 2.0 and 3.0 is an indication that bacterial cells are at an optimal density for Harvesting, and plasmid DNA isolation.

IMPORTANT: DO NOT EXCEED MAXIMUM CULTURE VOLUMES!

High-copy-number maximum culture volume = 15ml

2. Harvest the bacterial cells by centrifugation at 10,000 x g for 1 min at room temperature.
3. Decant or aspirate medium. To ensure that all traces of the medium are removed, use a clean paper towel to blot excess liquid from the wall of the vessel.
4. Add 500 μ l Solution I/RNase A to the bacterial pellet. Resuspend cells completely by vortexing or pipetting. Transfer the resuspended cells to a sterile 1.5 ml microcentrifuge tube.

NOTE: Complete resuspension of cell pellet is vital for obtaining good

yields.

DO NOT VORTEX FOLLOWING STEP 4 , DOING SO WILL SHEAR CHROMOSOMAL DNA.

5. Add 500µl Solution II and mix GENTLY but thoroughly by inverting and rotating the tube 4-6 times to obtain a cleared lysate. A 1-2 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.)
6. Add 250µl ice-cold Buffer N3 and mix GENTLY but thoroughly by inverting the tube several times until a flocculent white precipitate forms. Centrifuge at maxi speed ($\geq 13,000 \times g$) for 10 minutes at room temperature
7. Add Equal volume of ETR Binding Buffer to the cleared lysate. Mix thoroughly by inverting the tube 10 times.
8. Take a HiBind® DNA Mini Column pre-inserted in a 2ml collection tube. Add 100 µl Equilibration Buffer into the column. Centrifuge at 12,000 x g for 1 minutes.
9. Add 700 µl of the cleared lysate from step 7 into the Mini column and centrifuge at 13,000 x g for 1 minutes. Discard the flow-through and re-use the collection tube.
10. Repeat step 9 until all of the cleared lysate pass through the Mini column.
11. Add 500 µl of ETR Wash Buffer to the Mini column and centrifuge at 13,000 x for 30 seconds. Discard the flow-through and re-use the collection tube.
12. Add 500 µl of Buffer HB to the Midi column and centrifuge at 13,000 x g for 30 seconds. Discard the flow-through and re-use the collection tube.
13. Add 700 µl DNA Wash Buffer diluted with absolute ethanol to the Mini column and centrifuge at 13,000 x g for 30 seconds. Discard the flow-through and re-use the collection tube.

Note: DNA Wash Buffer must be diluted with absolute ethanol before use. See label for directions. If refrigerated, DNA Wash Buffer must be brought to the room temperature before use.
14. Add another 700 µl DNA Wash Buffer diluted with absolute to the Mini column and centrifuge at 13,000 x g for 30 seconds. Discard the flow-through and re-use the collection tube.

15. Centrifuge the empty column at 13,000 x g for 1 minute to dry the column matrix. Do not skip this step - it is critical for removing ethanol from the column.
16. Place column into a clean 1.5 ml centrifuge tube. Add 50-100 µl (depending on desired final concentration) of Endo-Free Elution Buffer (10 mM Tris, pH8.5) onto the column matrix and let it sit at room temperature for 2-3 minutes. Centrifuge at 13,000 x g) for 1 min to elute DNA. This represents approximately 60-80% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration. Alternatively, a second elution may be performed using the first eluate to maintain a high DNA concentration. Preheating the water to 65°C and allowing the column to soak 2 min at room temperature before elution may significantly increase yields.

Endo-Free Plasmid Mini Kit I Vacuum/Spin Protocol

Things to do before starting:

Pre-Chill Buffer N3 on ice

Preheat Elution Buffer to 70°C if Plasmid DNA is >10kb

Prepare DNA Wash Buffer and Solution1 according to directions on page 4

1. Prepare cell lysate by following step 1-7 from the spin protocol on page 8-9.
2. Prepare the vacuum manifold by following the manufacturer's instructions. Insert the HiBind® Mini DNA Column into a leur connector on the manifold.
3. Equilibrate the column: Add 100 µl Equilibration Buffer into the column, Turn on the vacuum to draw the liquid through the membrane. Add 100 µl water to the column and continue the vacuum until all the liquid pass through the column. Turn off the vacuum.
4. Pour the lysate from step 7 to the Mini column. Switch on the vacuum source to draw the solution through the column, keep adding the lysate until all the sample passes through the column and immediately switch off the vacuum source when lysate passes through the membrane .

IMPORTANT: It is important to keep the membrane wet for next wash step. If the membrane is dried, the flow rate will be significant reduced due to foaming of the detergent in the ETR Binding Buffer.
5. Add 500 µl ETR Wash Buffer to the column. Switch on the vacuum source to draw the solution through the column, and then switch off the vacuum source.
6. Apply 500 µl Buffer HB to the Mini column. Switch on the vacuum source to draw the solution through the column, and then switch off the vacuum source.
7. Apply 700 µl DNA Wash Buffer to the Mini column. Switch on the vacuum source to draw the solution through the column.
8. Apply another 700µl DNA Wash Buffer to the Mini column. Switch on the vacuum source to draw the solution through the column. After the solution has moved through the column, continue with the vacuum for another 10 minutes to dry the membrane. Switch off the vacuum source.

9. Place the column into a 2 ml centrifuge tube(provided). Centrifuge the empty column at 12,000 x g for 2 min to dry the column matrix. Do not skip this step - it is critical for removing ethanol from the column.
10. Place column into a clean 1.5 ml centrifuge tube. Add 50-100 µl (depending on desired final concentration)of Endo-Free Elution Buffer (10 mM Tris, pH8.5) onto the column matrix and let it sit at room temperature for 2-3 minutes. Centrifuge at 12,000 x g for 1 minute to elute DNA. An optional second elution will yield any residual DNA, though at a lower concentration. Alternatively, a second elution may be performed using the first eluate to maintain a high DNA concentration. Preheating the water to 65°C and allowing the column to soak 2 min at room temperature before elution may significantly increase yields.

Purification of plasmid DNA prepared by other methods

1. Bring up the volume of plasmid to 300 µl with water.
2. Adjust the binding conditions by adding equal volume of ETR Binding Buffer and mix thoroughly by vortexing briefly.
3. Apply the sample to the HiBind® DNA Mini Columns. Draw the sample through the membrane by centrifugation or vacuum.
4. Continue the appropriate protocol beginning the DNA Wash Buffer step and finishing with the Elution step.

Guidelines for Vac-8 manifold

Vac-8 manifold facilitate DNA preparations by providing a convenient modular vacuum manifold for use with HiBind® spin columns. The following recommendations should be followed when handling Vac-8 manifold:

1. Keep the Vac-8 manifold in a clean and dry condition. To clean, simply rinse all components with water and dry with absorbent paper. Do not use abrasive or solvents to clean the manifold.
2. The components of Vac-8 manifold are not resistant to ethanol, methanol, or other organic solvents. Do not bring solvent into contact with the vacuum manifold. Rinse the manifold with water thoroughly if solvents are spilled on the unit.

Yield and Quality of DNA

Determine the absorbance of an appropriate dilution (20- to 50-fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

$$\text{DNA concentration} = \text{Absorbance}_{260} \times 50 \times (\text{Dilution Factor}) \mu\text{g/ml}$$

A value greater than 1.8 indicates greater than 90% nucleic acid. 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. Typically, the majority of the DNA eluted is in monomeric supercoil form, though concatamers may also be present.

Troubleshooting Guide

Possible Problems and Suggestions

Low DNA yields

■ Poor Cell Lysis

Reduce the initial volume of culture or increase the lysis time while monitoring the lysis visually.

Cells may not have been dispersed adequately prior to the addition of Solution II. Make sure to vortex cell suspension to completely disperse.

Solution II if not tightly closed, may need to be replaced. Prepare as follows: 0.2 N NaOH, 1% SDS.

■ Bacterial Clone is overgrown or not fresh.

Do not incubate cultures for more than 24 hr at 37°C. Storage of cultures for extended periods prior to plasmid isolation is detrimental.

■ Low elution efficiency

If using Endotoxin-free water for elution, adjust the pH of water must be ≥ 8.0

■ Low copy-number plasmid used

Such plasmids may yield as little as 0.1µg of DNA from a 1 ml overnight culture. Double culture volume and follow suggested modifications with low copy number plasmid protocol.

■ Alkaline lysis is prolonged

Reduce the lysis time (Solution II) to 3 minutes or until the suspended cells form a clear viscous solution.

■ Too many or too few cells were used.

Confirm the cell density by measuring OD₆₀₀. To calculate the volume of culture to use, take the desired cell mass and divide by the absorbance of the overnight culture at 600 nm.

No DNA Eluted

■ DNA Wash Buffer not diluted with ethanol.

Prepare DNA Wash Buffer Concentrate according to instructions on page 4.

High molecular weight DNA contamination of product.

■ Over mixing of cell lysate upon addition of Solution II.

Do not vortex or mix aggressively after adding Solution II.

■ Culture overgrown

Overgrown culture contain lysed cells and degraded DNA. Do not grow cell longer than 16 hours.

Plasmid DNA floats out of well while loading agarose gel

■ Ethanol has not completely been removed from column following wash steps.

Centrifuge column as instructed to dry the column before elution

Absorbance of purified DNA does not accurately reflect quantity of the plasmid (A₂₆₀/A₂₈₀ ratio is high or low).

■ DNA Wash Buffer is diluted with ethanol containing impurities.

Check the absorbance of the ethanol between 250nm and 300nm. Do not use ethanol with high absorbance. Traces of impurities may remain on the binding column after washing and contribute to the absorbance in the final product.

■ Plasmid DNA is contaminated with RNA; RNase A treatment is insufficient.

Confirm that the RNase A Solution was added to Solution I prior to first use. The RNase A Solution may degrade due to high temperatures (>65°C) or prolonged storage (> 6 months at room temperature)

■ Background reading is high due to silica fines.

Spin the DNA sample at maximum speed for 1 minutes; use the supernatant to repeat the absorbance readings.

■ Purification is incomplete due to column overloading.

Reduce the initial volume of culture.

■ Plasmid DNA is contaminated with chromosomal DNA

Do not use cultures that have grown for more than 24 hours or are in the cell death phase. Do not vortex or vigorously shake the cells during the lysis reaction or N3 procedure.