E.Z.N.A.® HP Plasmid DNA Mini Kit I
E.Z.N.A.® HP Plasmid DNA Mini Kit II

Table of Contents

Manual Revision: July 2013

OMEGA bio-tek

Innovations in nucleic acid isolation

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 Omega Bio-tek's proprietary HiBind® matrix that avidly, but reversibly, binds DNA or RNA under optimized conditions, allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or low salt buffer.

The E.Z.N.A.® HP Plasmid DNA Mini Kits combine the power of HiBind® technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high-quality DNA in less than 20 minutes. Omega Bio-tek's HiBind® DNA 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 growth conditions, but a 5 mL overnight culture in LB medium typically yields 15-25 µg plasmid DNA with the E.Z.N.A.® HP Plasmid DNA Mini Kit I (D7042/D7043). The E.Z.N.A.® HP Plasmid DNA Mini Kit II (D7045) uses the same mini column format but is scaled up for isolation of low copy number plasmids and typically yields 40-75 µg DNA from 10-15 mL culture when using high copy plasmids. E.Z.N.A.® HP Plasmid DNA Mini Kit I is a simple, efficient, and fast to facilitate screening of recombinant clones. Whereas, the E.Z.N.A.® HP Plasmid DNA Mini Kit II employs an additional wash step that produces high-quality DNA suitable for automated fluorescent DNA sequencing, restriction endonuclease digestion, transfection of mammalian cells, and other applications.

The E.Z.N.A.® HP plasmid DNA purification system is the modified version of the E.Z.N.A.® plasmid isolation system; it is specially designed for those applications in which high-quality plasmids are required, such as transfection, autosequencing, etc. It is also suitable for isolating plasmids from bacterial hosts with high levels of endonuclease activity (such as EndoA+ strains). The plasmids from this system have excellent stability for long-term storage.

New in this Edition:

- This manual has been edited for content and redesigned to enhance user readability.
- HB Buffer has been replaced by HBC Buffer. Isopropanol is required and supplied by the user.
- Equilibration Buffer is no longer included with this kit. An optional Column Equilibration Protocol has been added to the protocol for your convenience.
- Equilibration Buffer is replaced with 3M NaOH provided by the user.

Storage and Stability

All of the E.Z.N.A.® HP Plasmid DNA Mini Kit I and HP Plasmid DNA Mini Kit II components are guaranteed for at least 24 months from the date of purchase when stored as follows. Solution I (once RNase A is added) should be stored at 2-8˚C. OB Protease Solution should be stored at -20°C. All other materials should be stored at room temperature. Solution II must be tightly capped when not in use.

Kit Contents

HP Plasmid Mini Kit I	D7042-00 D7043-00	D7042-01 D7043-01	D7042-02 D7043-02
Preps	5	50	200
HiBind® DNA Mini 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	30 ml	2 x 40 ml
HBC Buffer	4 mL	25 mL	80 mL
DNA Wash Buffer	1.5 ml	15 ml	3 x 25 ml
RNase A	50 µl	100 µl	400 µl
OB Protease Solution	70 µl	550 µl	2.1 ml
Elution Buffer	1.5 mL	15 mL	50 mL
User Manual	✓	✓	✓

HP Plasmid Mini Kit II	D7045-00	D7045-01	D7045-02
Preps	5	50	200
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
Solution III	5 mL	40 mL	2 x 80 mL
HBC Buffer	4 mL	25 mL	80 mL
DNA Wash Buffer	1.5 mL	15 mL	3 x 25 mL
RNase A	50 µL	150 µL	400 µL
OB Protease Solution	70 µl	550 µl	2.1 ml
Elution Buffer	1.5 mL	15 mL	50 mL
User Manual	✓	✓	✓

Preparing Reagents

1. Add the vial of RNase A to the bottle of Solution I and store at 2-8˚C.

2. Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D7042-00 **D7043-00** **D7045-00**	6 mL
D7042-01 **D7043-01** **D7045-01**	60 mL
D7042-02 **D7043-02** **D7045-02**	100 mL

3. Dilute HBC Buffer with isopropanol as follows and store at room temperature.

Kit	Isopropanol to be Added
D7042-00 **D7043-00** **D7045-00**	1.6 mL
D7042-01 **D7043-01** **D7045-01**	10 mL
D7042-02 **D7043-02** **D7045-02**	32 mL

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:

DNA concentration = Absorbance 260 × 50 × (Dilution Factor) µg/mL

A ratio greater than 1.8 indicates greater than 90% nucleic acid. Alternatively, quantity (as well as quality) sometimes can be determined best 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 concatemers may also be present.

Plasmid Copy Number and Expected Yield

Yield and quality of the plasmid DNA obtained depends on a number of factors including plasmid copy number, size of insert, host strain, culture volume, culture medium, and binding capacity of the kit. Of these factors, the vector copy number, culture volume, and kit binding capacity are most important. Plasmid copy number ranges from one copy to several hundred copies per cell as dictated by their origin of replication. Very large plasmids often display a very low copy number per cell.

Column Specifications

Plasmid Kit	D7042 D7043	D7045
Maximum Volume	750 µL	750 µL
Plasmid Binding Capacity	30 µg	75 µg
Yield: from 5 mL cultures (high copy number) from 15 mL cultures (high copy number)	 20-25 µg 20-30 µg	 20-25 µg 60-70 µg
Applications: Screening Minipreps DNA Sequencing Subcloning - enzymatic reactions Transfections	 ✓ ✓ ✓ ✓	 ✓ ✓ ✓ ✓

Guidelines for Vacuum Manifold

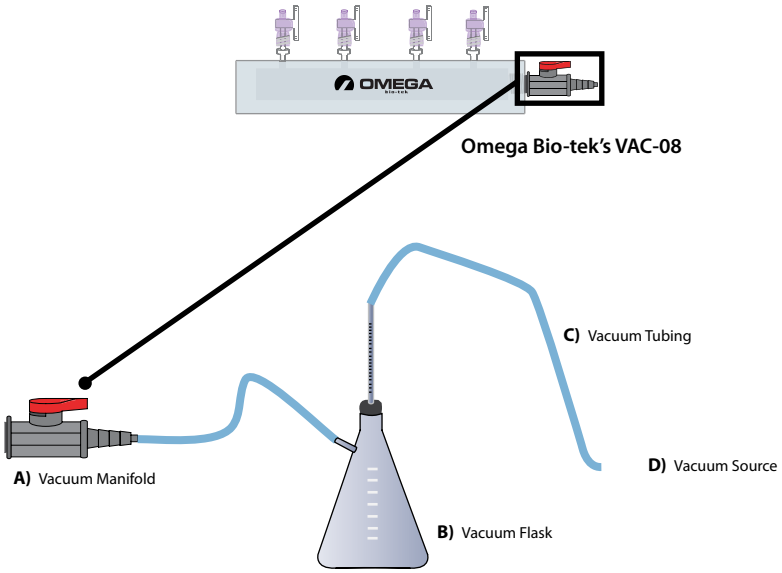
The following is required for use with the Vacuum/Spin Protocol:

A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-08)
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma AldrichVM20, Promega Vacman®, or manifold with standard Luer connector
B) Vacuum Flask
C) Vacuum Tubing
D) Vacuum Source (review tables below for pressure settings)

Manifold	Recommended Pressure (mbar)
VAC-08	-200 to -600

Conversion from millibars:	Multiply by:
Millimeters of mercury (mmHg)	0.75
Kilopascals (kPa)	0.1
Inches of mercury (inch Hg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Vacuum Setup:



E.Z.N.A.® HP Plasmid DNA Mini Kit I - Centrifugation Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- Sterile deionized water
- 100% ethanol
- Isopropanol
- Optional: 3M NaOH

Before Starting:

- Prepare DNA Wash Buffer, HBC Buffer, OB Protease Mixture, and Solution I according to the instructions in the Preparing Reagents section on Page 4

1. Isolate a single colony from a freshly streaked selective plate, and inoculate a culture of 5 mL LB medium containing the appropriate selective antibiotic. Incubate for ~12-16 hours at 37°C with vigorous shaking (~ 300 rpm). It is strongly recommended that an endA negative strain of *E. coli* be used for routine plasmid isolation. Examples of such strains include DH5a® and JM109®.

2. Centrifuge at 10,000 x *g* for 1 minute at room temperature.

3. Decant or aspirate and discard the culture media.

4. Add 250 µL Solution I/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.

   Note: RNase A must be added to Solution I before use. Please see the instructions in the Preparing Reagents section on Page 4.

5. Add 250 µL Solution II and 10 µL OB Protease Solution. Invert and gently rotate the tube several times to obtain a clear lysate. A 3-5 minute incubation may be necessary.

   Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution II tightly capped when not in use to avoid acidification from CO_2 in the air.

6. Add 350 µL Solution III. Immediately invert several times until a flocculent white precipitate forms.

   Note: It is vital that the solution is mixed thoroughly and immediately after the addition of Solution III to avoid localized precipitation.

7. Centrifuge at maximum speed (≥13,000 x *g*) for 10 minutes. A compact white pellet will form. Promptly proceed to the next step.

8. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Centrifuge at maximum speed for 30-60 seconds.
3. Discard the filtrate and reuse the collection tube.

9. Transfer the cleared supernatant from Step 7 by CAREFULLY aspirating it into the HiBind® DNA Mini Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Mini Column.

10. Centrifuge at maximum speed for 1 minute.

11. Discard the filtrate and reuse the collection tube.

12. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.

13. Centrifuge at maximum speed for 1 minute.

14. Discard the filtrate and reuse collection tube.

15. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

16. Centrifuge at maximum speed for 1 minute.

17. Discard the filtrate and reuse the collection tube.

Optional: Repeat Steps 15-17 for a second DNA Wash Buffer wash step.

18. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.

    Note: It is important to dry the HiBind® DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

19. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

20. Add 50-100 µL Elution Buffer or sterile deionized water directly to the center of the column membrane.

    Note: The efficiency of eluting DNA from the HiBind® DNA Mini Column is dependent on pH. If using sterile deionized water, make sure that the pH is around 8.5.

21. Centrifuge at maximum speed for 1 minute.

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

22. Store DNA at -20°C.

E.Z.N.A.® HP Plasmid DNA Mini Kit I - Vacuum Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- Vacuum Manifold
- Nuclease-free 1.5 mL microcentrifuge tubes
- Sterile deionized water
- 100% ethanol
- Isopropanol
- Optional: 3M NaOH

Before Starting:

- Prepare DNA Wash Buffer, HBC Buffer, and Solution I according to the instructions in the Preparing Reagents section on Page 4

1. Isolate a single colony from a freshly streaked selective plate, and inoculate a culture of 5 mL LB medium containing the appropriate selective antibiotic. Incubate for ~12-16 hours at 37°C with vigorous shaking (~ 300 rpm). It is strongly recommended that an endA negative strain of *E. coli* be used for routine plasmid isolation. Examples of such strains include DH5a® and JM109®.

2. Centrifuge at 10,000 x *g* for 1 minute at room temperature.

3. Decant or aspirate and discard the culture media.

4. Add 250 µL Solution I/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.

   Note: RNase A must be added to Solution I before use. Please see the instructions in the Preparing Reagents section on Page 4.

5. Add 250 µL Solution II and 10 µL OB Protease Solution. Invert and gently rotate the tube several times to obtain a clear lysate. A 3-5 minute incubation may be necessary.

   Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution II tightly capped when not in use to avoid acidification from CO_2 in the air.

6. Add 350 µL Solution III. Immediately invert several times until a flocculent white precipitate forms.

   Note: It is vital that the solution is mixed thoroughly and immediately after the addition of Solution III to avoid localized precipitation.

7. Centrifuge at maximum speed (≥13,000 x *g*) for 10 minutes. A compact white pellet will form. Promptly proceed to the next step.

8. Prepare the vacuum manifold according to manufacturer's instructions.

9. Connect the HiBind® DNA Mini Column to the vacuum manifold.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Turn on the vacuum source to draw the NaOH through the column.
3. Turn off the vacuum.

10. Transfer the cleared supernatant from Step 7 by CAREFULLY aspirating it into the HiBind® DNA Mini Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Mini Column.

11. Turn on the vacuum source to draw the sample through the column.

12. Turn off the vacuum.

13. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.

14. Turn on the vacuum source to draw the buffer through the column.

15. Turn off the vacuum.

16. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

17. Turn on the vacuum source to draw the buffer through the column.

18. Turn off the vacuum.

19. Repeat Steps 16-18 for a second DNA Wash Buffer wash step.

20. Transfer the HiBind® DNA Mini Column to a 2 mL Collection Tube.

21. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.

    Note: It is important to dry the HiBind® DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

22. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

23. Add 50-100 µL Elution Buffer or sterile deionized water directly to the center of the column membrane.

    Note: The efficiency of eluting DNA from the HiBind® DNA Mini Column is dependent on pH. If using sterile deionized water, make sure that the pH is around 8.5.

24. Centrifuge at maximum speed for 1 minute.

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

25. Store DNA at -20°C.

E.Z.N.A.® HP Plasmid DNA Mini Kit II - Centrifugation Protocol

Note: Using the following protocol with Cat. No. D7042/D7043 will not improve yields significantly with high copy plasmids due to the lower column binding capacity (see column specifications on Page 5).

The E.Z.N.A.® HP Plasmid DNA Mini Kit II allows rapid and reliable isolation of greater than 50 µg plasmid DNA using the spin-column format. There is no need for organic extractions or alcohol precipitations, and the purified DNA is suitable for many downstream applications including double stranded DNA sequencing.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- Centrifuge capable of 5,000 x *g* preferably with a swing bucket rotor
- Nuclease-free 2 mL microcentrifuge tubes
- Sterile deionized water
- 100% ethanol
- Isopropanol
- Optional: 3M NaOH

Before Starting:

- Prepare DNA Wash Buffer, HBC Buffer, OB Protease Mixture, and Solution I according to the instructions in the Preparing Reagents section on Page 4

1. Isolate a single colony from a freshly streaked selective plate, and inoculate a culture of 10-15 mL LB medium containing the appropriate selective antibiotic. Incubate for ~12-16 hours at 37°C with vigorous shaking (~ 300 rpm). It is strongly recommended that an endA negative strain of *E. coli* be used for routine plasmid isolation. Examples of such strains include DH5a® and JM109®. For low-copy plasmids use no more than 25 mL medium.

2. Centrifuge at 5,000 x *g* for 10 minutes at room temperature. Use a swing bucket centrifuge if available.

3. Decant or aspirate and discard the culture media.

4. Add 500 µL Solution I/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.

   Note: RNase A must be added to Solution I before use. Please see the instructions in the Preparing Reagents section on Page 4.

5. Transfer the sample to a clean 2 mL microcentrifuge tube.

6. Add 500 µL Solution II and 10 µL OB Protease Solution. Invert and gently rotate the tube several times to obtain a clear lysate. A 3-5 minute incubation may be necessary.

   Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution II tightly capped when not in use to avoid acidification from CO_2 in the air.

7. Add 700 µL Solution III. Immediately invert several times until a flocculent white precipitate forms.

   Note: It is vital that the solution is mixed thoroughly and immediately after the addition of Solution III to avoid localized precipitation.

8. Centrifuge at maximum speed (≥13,000 x *g*) for 10 minutes. A compact white pellet will form. Promptly proceed to the next step.

9. Insert a HiBind® DNA Mini Column II into a 2 mL Collection Tube.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column II.
2. Centrifuge at maximum speed for 30-60 seconds.
3. Discard the filtrate and reuse the collection tube.

10. Transfer the 700 µL cleared supernatant from Step 8 by CAREFULLY aspirating it into the HiBind® DNA Mini Column II. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Mini Column II.

11. Centrifuge at maximum speed for 1 minute.

12. Discard the filtrate and reuse the collection tube.

13. Repeat Steps 10-12 until all the remaining samples has been transferred to the HiBind® DNA Mini Column II.

14. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.

15. Centrifuge at maximum speed for 1 minute.

16. Discard the filtrate and reuse collection tube.

17. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

18. Centrifuge at maximum speed for 1 minute.

19. Discard the filtrate and reuse the collection tube.

Optional: Repeat Steps 17-19 for a second DNA Wash Buffer wash step.

20. Centrifuge the empty HiBind® DNA Mini Column II for 2 minutes at maximum speed to dry the column matrix.

    Note: It is important to dry the HiBind® DNA Mini Column II matrix before elution. Residual ethanol may interfere with downstream applications.

21. Transfer the HiBind® DNA Mini Column II to a clean 1.5 mL microcentrifuge tube.

22. Add 100-200 µL Elution Buffer or sterile deionized water directly to the center of the column membrane.

    Note: The efficiency of eluting DNA from the HiBind® DNA Mini Column II is dependent on pH. If using sterile deionized water, make sure that the pH is around 8.5.

23. Centrifuge at maximum speed for 1 minute.

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

24. Store DNA at -20°C.

E.Z.N.A.® Plasmid Mini Kit Protocol - Low Copy Number Plasmid DNA Protocol

Low copy number plasmids generally give 0.1-0.5 µg DNA per mL overnight culture. For the isolation of plasmid DNA from low copy number plasmids (0.1-1 µg/mL culture) or low copy number plasmid (1-2 µg/mL culture) bacteria, use the following modified protocol.

Note: The E.Z.N.A.® HP Plasmid DNA Mini Kit I and the E.Z.N.A.® HP Plasmid DNA Mini Kit II come with enough Solution I, Solution II, and Solution III to perform the standard protocols. Additional Solution I, Solution II, and Solution III are needed to perform the Low Copy Number Plasmid DNA Protocol. These buffers can be purchased separately. See Page 21 for ordering information.

Note: This method is not recommended for high copy number plasmids because with culture volumes greater than 5 mL, the HiBind® DNA Mini Column quickly becomes saturated. In this situation, we recommend processing multiple samples from the same culture. Alternatively, use the E.Z.N.A.® HP Plasmid DNA Mini Kit II (Cat. No. D7045) that allows processing of 10-15 mL cultures using the mini column format and generally yields up to 70 µg plasmid DNA with high copy plasmids.

1. Increase the volume of starting culture from that of high copy number plasmids. Use 5-10 mL bacterial culture for the E.Z.N.A.® Plasmid DNA Mini Kit I or 20-30 mL bacterial culture for E.Z.N.A.® Plasmid DNA Mini Kit II.

2. Pellet the bacterial cells by centrifugation.

3. Decant or aspirate and discard the culture media.

4. Perform Steps 4-6 of the E.Z.N.A.® HP Plasmid DNA Kit I protocols or Steps 4-7 of the E.Z.N.A.® HP Plasmid DNA Kit II protocol with **double the volumes of Solution I, Solution II, and Solution III.**

5. Continue with Step 7 of the E.Z.N.A.® HP Plasmid DNA Kit I protocols or Step 8 of the E.Z.N.A.® HP Plasmid DNA Kit II protocol by following the wash, drying, and elution steps. **There is no need to increase the volumes of HBC Buffer, DNA Wash Buffer, or Elution Buffer.**

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Low DNA yields	Poor cell lysis	Only use LB or YT medium containing ampicillin. Do not use more than 5 mL (high copy plasmids) or 10 mL (low copy plasmids) culture with the basic protocols. Increase Solution II/OB Protease incubation time in Step 5. Cells may not be dispersed adequately prior to addition of Solution II. Vortex cell suspension to completely disperse. Solution II if not tightly closed, may need to be replaced.
	Bacterial culture overgrown or not fresh	Do not incubate cultures for more than 16 hr at 37°C. Storage of cultures for extended periods prior to plasmid isolation is detrimental.
	Low copy number plasmid used	Such plasmids may yield as little as 0.5 µg DNA from a 5 mL overnight culture. Increase culture volume to 10 mL and follow suggested modifications with Cat. No. D7043 or use the HP Plasmid DNA Mini Kit II with 25 mL culture.
	Column matrix lost binding capacity during storage	Follow the **Optional Protocol for Column Equilibration** prior to transferring the cleared lysate to the HiBind® DNA Mini Column. Add 100 µL 3M NaOH to the column prior to loading the sample. Centrifuge at 10,000 x *g* for 30 seconds. Discard the filtrate.
Problem	Cause	Solution
No DNA eluted	DNA Wash Buffer not diluted with 100% ethanol	Prepare DNA Wash Buffer according to instructions on Page 4.
	HBC Buffer not diluted with isopropanol	Prepare HBC Buffer according to instructions on Page 4.

Troubleshooting Guide

Problem	Cause	Solution
High molecular weight DNA contamination	Over mixing of cell lysate upon addition of Solution II	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 increase A_{260}	Make sure to wash the column as instructed. Repeat the DNA Wash Buffer Wash step. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantification.
RNA visible on agarose gel	RNase A not added to Solution I	Add the RNase to Solution I.
Plasmid DNA floats out of well while loading agarose gel	Ethanol was not completely removed from column following wash steps	Centrifuge column as instructed to dry the column before elution.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
DNase/RNase-free microcentrifuge tubes, 1.5 mL, 500/pk, 10 pk/cs	SSI-1210-00
DNase/RNase-free microcentrifuge tubes, 2.0 mL, 500/pk, 10 pk/cs	SSI-1310-00
Vacuum Manifold	VAC-08
HiBind® DNA Mini Columns (200)	DNACOL-02
Solution I (250 mL)	PS001
Solution II (250 mL)	PS002
Solution III (250 mL)	PS003
Elution Buffer (100 mL)	PDR048
DNA Wash Buffer (100 mL)	PS010
RNase A (400 µL)	AC117

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PCR is a patented process of Hoffman-La Roche. Use of the PCR process requires a license.