omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® Plasmid DNA Midi Kit

D6904-00	2 preps
D6904-03	25 preps
D6904-04	100 preps

E.Z.N.A.® Plasmid DNA Maxi Kit

D6922-00	2 preps
D6922-02	20 preps
D6922-04	100 preps

Manual Date: September 2021
Revision Number: v4.2

For Research Use Only

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E.Z.N.A.® Plasmid DNA Midi Kit
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Table of Contents

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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 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.® Plasmid DNA Midi and Maxi Kits combine the power of HiBind® technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high-quality DNA. HiBind® DNA columns facilitate the binding, washing, and elution steps thus enabling multiple samples to be processed simultaneously. Following lysis, the DNA is bound to the silica membrane and contaminants are removed with a few simple wash steps. Plasmid DNA is eluted with low salt buffer. Plasmid DNA purified by this system is suitable for automated fluorescent DNA sequencing, restriction endonuclease digestion, transfection of mammalian cells, and other manipulations.

Yields vary according to plasmid copy number, *E. coli* strain, and growth conditions.

Up to 100-250 µg high copy number plasmid DNA or 10-50 µg low copy number plasmid DNA can be purified from 20-50 mL overnight culture using the E.Z.N.A.® Plasmid DNA Midi Kit.

Up to 600-1200 µg high copy number plasmid DNA or 50-300 µg low copy number plasmid DNA can be purified from 50-200 mL overnight culture using E.Z.N.A.® Plasmid DNA Maxi Kit.

New In this Edition:

- HB Buffer has been replaced by HBC Buffer.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.® Plasmid DNA Midi and Maxi Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. After RNase A is added, Solution I should be stored at 2-8˚C. During shipment or storage in cool ambient conditions, precipitates may form in HBC Buffer, Solution II, and Solution III. Dissolve such deposits by warming the solution at 37°C and gently shaking.

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) 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, supercoiled form, though concatemers may also be present.

Plasmid Copy Number and Expected Yield

The 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 kits. 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. But very large plasmids often display a very low copy number per cell. The expected yield of 50 mL overnight cultures (LB medium) with the E.Z.N.A.® Plasmid DNA Midi or Maxi Kit are indicated in the following table.

Sample yields from 50 mL starting culture

Plasmid	Replicon	Copy Number	Expected Yield (50 mL culture)
pUC vectors	pMBI	500-700	150-250 µg
pBluescript® vectors	ColE14	300-500	100-180 µg
pGEM® vectors	pMB1	300-400	100-200 µg
pBR322 and its derivatives	pMB1	15-20	10-20 µg
ColE14	ColE14	15-20	10-20 µg
PACYC and its derivatives	p15A	37-40	5-10 µg
pSC101 and its derivatives	pSC101	~5	5 µg
pGEM	pMB1	300-700	100-200 µg

Centrifugation Protocol

Pellet by Centrifugation

Resuspend
Lyse
Neutralize

Clear Lysate

Transfer cleared lysate to HiBind® DNA Column

Bind

Wash 3X

Dry

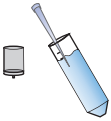
Elute

Vacuum Protocol

Pellet by Centrifugation

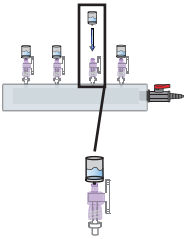
Resuspend
Lyse
Neutralize

Clear Lysate



Transfer cleared lysate to HiBind® DNA Column

Bind



Wash 3X

Dry

Elute

Kit Contents

E.Z.N.A.® Plasmid DNA Midi Kit	D6904-00	D6904-03	D6904-04
Purifications	2	25	100
HiBind® DNA Midi Columns	2	25	100
15 mL Collection Tubes	2	25	100
Solution I	8 mL	70 mL	270 mL
Solution II	8 mL	70 mL	270 mL
Solution III	8 mL	100 mL	400 mL
HBC Buffer	5 mL	58 mL	250 mL
DNA Wash Buffer	5 mL	40 mL	200 mL
RNase A	25 µL	300 µL	1.2 mL
Elution Buffer	5 mL	60 mL	220 mL
User Manual	✓	✓	✓

E.Z.N.A.® Plasmid DNA Maxi Kit	D6922-00	D6922-02	D6922-04
Purifications	2	20	100
HiBind® DNA Maxi Columns	2	20	100
50 mL Collection Tubes	2	20	100
Solution I	30 mL	270 mL	2 x 675 mL
Solution II	30 mL	270 mL	2 x 675 mL
Solution III	35 mL	2 x 170 mL	2 x 850 mL
HBC Buffer	25 mL	2 x 80 mL	3 x 250 mL
DNA Wash Buffer	25 mL	3 x 50 mL	4 x 200 mL
RNase A	100 µL	1.2 mL	2 x 3.0 mL
Elution Buffer	15 mL	250 mL	2 x 500 mL
User Manual	✓	✓	✓

Preparing Reagents

1. Add vial of RNase A to the bottle of Solution I provided 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
D6904-00	20 mL
D6904-03	160 mL
D6904-04	800 mL

Kit	100% Ethanol to be Added
D6922-00	100 mL
D6922-02	200 mL per bottle
D6922-04	800 mL per bottle

3. Dilute HBC Buffer with 100% isopropanol as follows and store at room temperature.

Kit	100% Isopropanol to be Added
D6904-00	2 mL
D6904-03	23 mL
D6904-04	100 mL

Kit	100% Isopropanol to be Added
D6922-00	10 mL
D6922-02	32 mL
D6922-04	100 mL per bottle

4. Check Solution II , Solution III, and HBC Buffer for precipitation before use. Redissolve any precipitation by warming to 37˚C.

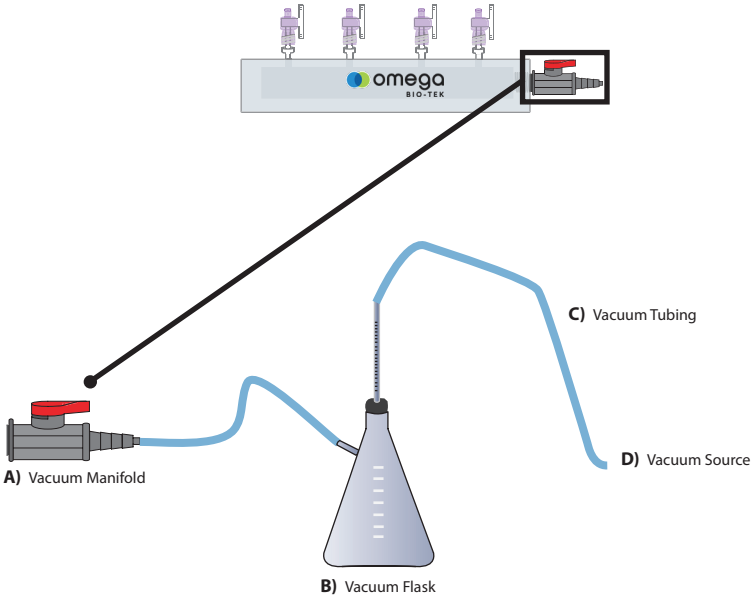
Guidelines for Vacuum Manifold

The following is required for use with the Vacuum Protocol:

A) 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)

Conversion from millibars:	Multiply by:
Millimeters of mercury (mm Hg)	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

Illustrated Vacuum Setup:



Growth and Culture of Bacteria

Bacterial Strain Selection

It is strongly recommended that an end A negative strain of *E. coli* be used for routine plasmid isolation. Examples of such strains include DH5α™ , DH1, and C600. These host strains yield high-quality DNA with E.Z.N.A.® Plasmid DNA Kits' 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, Solution II, and Solution 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 (~300 rpm; 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 DNA 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® DNA 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 OD_{600} is recommended. When using nutrient-rich media, care should be taken to ensure that the cell density does not exceed an OD_{600} of 3.0. Using a high-density culture outside of the recommended OD range may overload the purification system.

E.Z.N.A.® Plasmid DNA Midi Kit - Vacuum Protocol

All centrifugation steps should be carried out at room temperature unless otherwise noted. To improve yields of low copy number plasmid DNA, please see suggestions for "Low Copy Number Plasmids and Cosmids" on Page 27.

Materials and Equipment to be Supplied by User:

- Centrifuge with swing bucket rotor capable of 4,000*g*
- Centrifuge capable of 15,000*g*
- Vacuum manifold
- Nuclease-free 15 mL and 50 mL centrifuge tubes
- 30 mL or 50 mL centrifuge tubes capable of withstanding 15,000*g*
- 100% ethanol (do not use denatured alcohol)
- 100% isopropanol
- Optional: Water bath, incubator, or heat block capable of 65°C
- Optional: Sterile deionized water
- Optional: 3M NaOH

Before starting:

- Check Solution II and Solution III for precipitation before use. Redissolve any precipitates by warming to 37°C
- Prepare DNA Wash Buffer, HBC Buffer, and Solution I according to "Preparing Reagents" section on Page 6
- Optional: Heat Elution Buffer to 65°C if plasmid DNA is >10 kb

1. Transfer 20-50 mL overnight culture to a 50 mL centrifuge tube (not provided).

   Note: The optimal volume to use depends on the culture density and plasmid copy number. The optimal cell mass (OD_{600} x mL culture) for the HiBind® DNA Midi Column is 80-100. For example, if the OD_{600} of a culture is 4.0, the optimal culture volume should be 20-25 mL. If excess culture cell mass is used, alkaline lysis will be inefficient, the HiBind® membrane will be overloaded, and the performance of the system will be decreased. The increase in lysate viscosity will require vigorous mixing which may result in shearing of genomic DNA and contamination the plasmid DNA. For low copy number plasmids, see the "Low Copy Number Plasmids" protocol on Page 27.

2. Centrifuge at 4,000*g* for 10 minutes at room temperature.
3. Decant or aspirate and discard the culture media.

Note: To ensure that all traces of the medium are removed, use a clean paper towel to blot excess liquid from the wall of the tube.

4. Add 2.25 mL Solution I/RNase A. Vortex or pipet up and down to completely resuspend the cells.

   Note: RNase A must be added to Solution I before use. Please see the instructions in the "Preparing Reagents" section on Page 6.

5. Transfer the cell suspension to a 30 mL or 50 mL centrifuge tubes capable of withstanding 15,000*g* (not provided).

6. Add 2.25 mL Solution II. Invert and rotate the tube gently 8-10 times to obtain a cleared lysate. This may require a 2-3 minute incubation at room temperature with occasional mixing.

   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 3.2 mL Solution III. Invert and rotate the tube gently until flocculent white precipitates form. This may require a 2-3 minute incubation at room temperature with occasional mixing.

   Note: It is vital that the solution is mixed thoroughly and immediately after the addition of Solution III to avoid localized precipitation. If the mixture still appears viscous, brownish, or conglobated, more mixing is required to completely neutralize the solution.

8. Centrifuge at 15,000*g* for 10 minutes at room temperature (preferably at 4°C). A compact white pellet will form.

9. Prepare the vacuum manifold by following the manufacturer's instructions.

10. Connect the HiBind® DNA Midi Column to the vacuum manifold. Refer to the Illustrated Vacuum Set Up on Page 7 for details.

Optional Protocol for Column Equilibration:

1. Add 1 mL 3M NaOH to the HiBind® DNA Midi Column.
2. Let sit for 2 minutes at room temperature.
3. Turn on the vacuum source to draw the NaOH through the column.
4. Turn off the vacuum.

11. Transfer 3.5 mL cleared supernatant from Step 8 by CAREFULLY pipetting it into the HiBind® DNA Midi Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Midi Column.

12. Turn on the vacuum source to draw the supernatant through the column.

13. Turn off the vacuum.

14. Repeat Steps 11-13 until all of the cleared supernatant has been transferred to the HiBind® DNA Midi Column.

15. Add 3 mL HBC Buffer.

    Note: HBC Buffer must be diluted with 100% isopropanol prior to use. Please see the instructions in the "Preparing Reagents" section on Page 6.

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

17. Turn off the vacuum.

18. Add 3.5 mL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see the instructions in the "Preparing Reagents" section on Page 6.

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

20. Turn off the vacuum.
21. Repeat Steps 18-20 for a second DNA Wash Buffer wash step.

22. Transfer the HiBind® DNA Midi Column to a 15 mL Collection Tube (provided).

23. Centrifuge the empty HiBind® DNA Midi Column at 4,000*g* for 2 minutes to dry the column matrix.

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

24. Transfer the HiBind® DNA Midi Column to a nuclease-free 15 mL centrifuge tube (not provided).

25. Add 0.5-1 mL Elution Buffer or sterile deionized water directly to the center of the column matrix.

26. Let it sit for 3 minutes at room temperature.

27. Centrifuge at 4,000*g* for 5 minutes.

    Note: This represents approximately 65-80% 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.

28. Store DNA at -20°C.

E.Z.N.A.® Plasmid DNA Midi Kit - Centrifugation Protocol

All centrifugation steps after Step 8 should be performed with a swing bucket rotor for maximum plasmid DNA yields. All centrifugation steps should be carried out at room temperature unless otherwise noted. To improve yields of low copy number plasmid DNA, please see suggestions for "Low Copy Number Plasmids and Cosmids" on Page 27.

Materials and Equipment to be Supplied by User:

- Centrifuge with swinging bucket rotor capable of 4,000*g*
- Centrifuge capable of 15,000*g*
- Nuclease-free 15 mL and 50 mL centrifuge tubes
- 30 mL or 50 mL centrifuge tubes capable of withstanding 15,000*g*
- 100% Ethanol (do not use denatured alcohol)
- 100% isopropanol
- Optional: Water bath, incubator, or heat block capable of 65°C
- Optional: Sterile deionized water
- Optional: 3M NaOH

Before starting:

- Check Solution II and Solution III for precipitation before use. Redissolve any precipitates by warming to 37°C
- Prepare DNA Wash Buffer, HBC Buffer, and Solution I according to "Preparing Reagents" section on Page 6
- Optional: Heat Elution Buffer to 65°C if plasmid DNA is >10 kb

1. Transfer 20-50 mL overnight culture to a 50 mL centrifuge tube (not provided).

   Note: The optimal volume to use depends on the culture density and plasmid copy number. The optimal cell mass (OD_{600} x mL culture) for the HiBind® DNA Midi Column is 80-100. For example, if the OD_{600} of a culture is 4.0, the optimal culture volume should be 20-25 mL. If excess culture cell mass is used, alkaline lysis will be inefficient, the HiBind® membrane will be overloaded, and the performance of the system will be decreased. The increase in lysate viscosity will require vigorous mixing which may result in shearing of genomic DNA and contamination the plasmid DNA. For low copy number plasmids, see the "Low Copy Number Plasmids" protocol on Page 27.

2. Centrifuge at 4,000*g* for 10 minutes at room temperature.
3. Decant or aspirate and discard the culture media.

Note: To ensure that all traces of the medium are removed, use a clean paper towel to blot excess liquid from the wall of the tube.

4. Add 2.25 mL Solution I/RNase A. Vortex or pipet up and down to completely resuspend the cells.

   Note: RNase A must be added to Solution I before use. Please see the instructions in the "Preparing Reagents" section on Page 6.

5. Transfer the cell suspension to a 30 mL or 50 mL centrifuge tubes capable of withstanding 15,000*g* (not provided).

6. Add 2.25 mL Solution II. Invert and rotate the tube gently 8-10 times to obtain a cleared lysate. This may require a 2-3 minute incubation at room temperature with occasional mixing.

   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 3.2 mL Solution III. Invert and rotate the tube gently until flocculent white precipitates form. This may require a 2-3 minute incubation at room temperature with occasional mixing.

   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 15,000*g* for 10 minutes at room temperature (preferably at 4°C). A compact white pellet will form. Promptly proceed to the next step.

Note: Steps 9-26 should be performed in a swing bucket rotor for maximum plasmid DNA yield. All of centrifugation steps should be carried out at room temperature.

9. Insert a HiBind® DNA Midi Column into a 15 mL Collection Tube (supplied).

Optional Protocol for Column Equilibration:

1. Add 1 mL 3M NaOH to the HiBind® DNA Midi Column.
2. Let sit for 4 minutes at room temperature.
3. Centrifuge at 4,000*g* for 3 minutes.
4. Discard the filtrate and reuse the collection tube.

10. Transfer 3.5 mL cleared supernatant from Step 8 by CAREFULLY pipettting it into the HiBind® DNA Midi Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Midi Column.

11. Centrifuge at 4,000*g* for 3 minutes.

12. Discard the filtrate and reuse the collection tube.

13. Repeat Steps 10-12 until all of the cleared supernatant has been transferred to the HiBind® DNA Midi Column.

14. Add 3 mL HBC Buffer.

    Note: HBC Buffer must be diluted with 100% isopropanol prior to use. Please see the instructions in the "Preparing Reagents" section on Page 6.

15. Centrifuge at 4,000*g* for 3 minutes.

16. Discard the filtrate and reuse the collection tube.

17. Add 3.5 mL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see the instructions in the "Preparing Reagents" section on Page 6.

18. Centrifuge at 4,000*g* for 3 minutes.

19. Discard the filtrate and reuse the collection tube.
20. Repeat Steps 17-19 for a second DNA Wash Buffer wash step.

21. Centrifuge the empty HiBind® DNA Midi Column at 4,000*g* for 10 minutes to dry the column matrix.

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

22. Transfer the HiBind® DNA Midi Column to a nuclease-free 15 mL centrifuge tube (not supplied).

23. Add 0.5-1 mL Elution Buffer or sterile deionized water directly to the center of the column matrix.

24. Let it sit for 3 minutes at room temperature.

25. Centrifuge at 4,000*g* for 5 minutes.

    Note: This represents approximately 65-80% 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.

26. Store DNA at -20°C.

E.Z.N.A.® Plasmid DNA Maxi Kit - Vacuum Protocol

All centrifugation steps should be carried out at room temperature unless otherwise noted. To improve yields of low copy number plasmid DNA, please see suggestions for "Low Copy Number Plasmids and Cosmids" on Page 27.

Materials and Equipment to be Supplied by User:

- Centrifuge with swing bucket rotor capable of 4,000*g*
- Centrifuge capable of 15,000*g*
- Vacuum manifold
- Nuclease-free 50 mL centrifuge tubes
- 30 mL or 50 mL centrifuge tubes capable of withstanding 15,000*g*
- Appropriate centrifuge bottle for Step 1
- 100% ethanol (do not use denatured alcohol)
- 100% isopropanol
- Optional: Water bath, incubator, or heat block capable of 65°C
- Optional: Sterile deionized water
- Optional: 3M NaOH

Before starting:

- Check Solution II and Solution III for precipitation before use. Redissolve any precipitates by warming to 37°C
- Prepare DNA Wash Buffer, HBC Buffer, and Solution I according to Preparing Reagents section on Page 6
- Optional: Heat Elution Buffer to 65°C if plasmid DNA is >10 kb

1. Transfer 50-200 mL overnight culture to an appropriate centrifuge bottle (not provided).

   Note: The optimal volume to use depends on the culture density and plasmid copy number. The optimal cell mass (OD_{600} x mL culture) for the HiBind® DNA Maxi Column is 300-400. For example, if the OD_{600} of a culture is 4.0, the optimal culture volume should be 75-100 mL. If excess culture cell mass is used, alkaline lysis will be inefficient, the HiBind® membrane will be overloaded, and the performance of the system will be decreased. The increase in lysate viscosity will require vigorous mixing which may result in shearing of genomic DNA and contamination the plasmid DNA. For low copy number plasmids, see the "Low Copy Number Plasmids" protocol on Page 27.

2. Centrifuge at 4,000*g* for 10 minutes at room temperature.

3. Decant or aspirate and discard the culture media.

   Note: To ensure that all traces of the medium are removed, use a clean paper towel to blot excess liquid from the wall of the bottle.

4. Add 12 mL Solution I/RNase A. Vortex or pipet up and down to completely resuspend the cells.

   Note: RNase A must be added to Solution I before use. Please see the instructions in the Preparing Reagents section on Page 6.

5. Transfer the cell suspension to a 30 mL or 50 mL centrifuge tubes capable of withstanding 15,000*g* (not provided).

6. Add 12 mL Solution II. Invert and rotate the tube gently 10-12 times to obtain a cleared lysate. This may require a 2-3 minute incubation at room temperature with occasional mixing.

   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 16 mL Solution III. Invert and rotate the tube gently until flocculent white precipitates form. This may require a 2-3 minute incubation at room temperature with occasional mixing.

   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 15,000*g* for 10 minutes at room temperature (preferably at 4°C). A compact white pellet will form. Promptly proceed to the next step.

9. Prepare the vacuum manifold by following the manufacturer's instructions.

10. Connect the HiBind® DNA Maxi Column to the vacuum manifold. Refer to the Illustrated Vacuum Set Up on Page 7 for details.

Optional Protocol for Column Equilibration:

1. Add 3 mL 3M NaOH to the HiBind® DNA Maxi Column.
2. Let sit for 4 minutes at room temperature.
3. Turn on the vacuum source to draw the NaOH through the column.
4. Turn off the vacuum.

11. Transfer 20 mL cleared supernatant from Step 8 by CAREFULLY pipetting it into the HiBind® DNA Maxi Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Maxi Column.

12. Turn on the vacuum source to draw the supernatant through the column.

13. Turn off the vacuum.

14. Repeat Steps 11-13 until all of the cleared supernatant has been transferred to the HiBind® DNA Maxi Column.

15. Add 10 mL HBC Buffer.

    Note: HBC Buffer must be diluted with 100% isopropanol prior to use. Please see the instructions in the "Preparing Reagents" section on Page 6.

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

17. Turn off the vacuum.

18. Add 15 mL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see the instructions in the "Preparing Reagents" section on Page 6.

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

20. Repeat Steps 17-19 for a second DNA Wash Buffer wash step.
21. Continue to apply the vacuum for 5 minutes to completely dry the HiBind® matrix.

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

22. Transfer the HiBind® DNA Maxi Column to a nuclease-free 50 mL centrifuge tube (not provided).

23. Add 1.5-3 mL Elution Buffer or sterile deionized water directly to the center of the column matrix.

24. Let it sit for 5 minutes at room temperature.

25. Centrifuge at 4,000*g* for 5 minutes.

    Note: This represents approximately 65-80% 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.

26. Store DNA at -20°C.

E.Z.N.A.® Plasmid DNA Maxi Kit - Centrifugation Protocol

All centrifugation steps after Step 8 should be performed with a swing bucket rotor for maximum plasmid DNA yields. All centrifugation steps should be carried out at room temperature unless otherwise noted. To improve yields of low copy number plasmid DNA, please see suggestions for "Low Copy Number Plasmids and Cosmids" on Page 27.

Materials and Equipment to be Supplied by User:

- Centrifuge with swing bucket rotor capable of 4,000*g*
- Centrifuge capable of 15,000*g*
- Nuclease-free 50 mL centrifuge tubes
- 30 mL or 50 mL centrifuge tubes capable of withstanding 15,000*g*
- Appropriate centrifuge bottle for Step 1
- 100% ethanol (do not use denatured alcohol)
- 100% isopropanol
- Optional: Water bath, incubator, or heat block capable of 65°C
- Optional: Sterile deionized water
- Optional: 3M NaOH

Before starting:

- Check Solution II and Solution III for precipitation before use. Redissolve any precipitates by warming to 37°C
- Prepare DNA Wash Buffer, HBC Buffer, and Solution I according to "Preparing Reagents" section on Page 6
- Optional: Heat Elution Buffer to 65°C if plasmid DNA is >10 kb

1. Transfer 50-200 mL overnight culture to an appropriate centrifuge bottle (not provided).

   Note: The optimal volume to use depends on the culture density and plasmid copy number. The optimal cell mass (OD_{600} x mL culture) for the HiBind® DNA Maxi Column is 300-400. For example, if the OD_{600} of a culture is 4.0, the optimal culture volume should be 75-100 mL. If excess culture cell mass is used, alkaline lysis will be inefficient, the HiBind® membrane will be overloaded, and the performance of the system will be decreased. The increase in lysate viscosity will require vigorous mixing which may result in shearing of genomic DNA and contamination the plasmid DNA. For low copy number plasmids, see the "Low Copy Number Plasmids" protocol on Page 27.

2. Centrifuge at 4,000*g* for 10 minutes at room temperature.

3. Decant or aspirate and discard the culture media.

   Note: To ensure that all traces of the medium are removed, use a clean paper towel to blot excess liquid from the wall of the tube.

4. Add 12 mL Solution I/RNase A. Vortex or pipet up and down to completely resuspend the cells.

   Note: RNase A must be added to Solution I before use. Please see the instructions in the Preparing Reagents section on Page 6.

5. Transfer the cell suspension to a 30 mL or 50 mL centrifuge tubes capable of withstanding 15,000*g* (not provided).

6. Add 12 mL Solution II. Invert and rotate the tube gently 10-12 times to obtain a cleared lysate. This may require a 2-3 minute incubation at room temperature with occasional mixing.

   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 16 mL Solution III. Invert and rotate the tube gently until flocculent white precipitates form. This may require a 2-3 minute incubation at room temperature with occasional mixing.

   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 15,000*g* for 10 minutes at room temperature (preferably at 4°C). A compact white pellet will form. Promptly proceed to the next step.

Note: Steps 9 -26 should be performed with a swing bucket rotor for maximum plasmid DNA yields. All of centrifugation steps should be carried out at room temperature.

9. Insert a HiBind® DNA Maxi Column into a 50 mL Collection Tube (provided).

Optional Protocol for Column Equilibration:

1. Add 3 mL 3M NaOH to the HiBind® DNA Maxi Column.
2. Let sit at room temperature for 4 minutes.
3. Centrifuge at 4,000*g* for 3 minutes.
4. Discard the filtrate and reuse the collection tube.

10. Transfer 20 mL cleared supernatant from Step 8 by CAREFULLY pipetting it into the HiBind® DNA Maxi Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Maxi Column.

11. Centrifuge at 4,000g for 5 minutes.

12. Discard the filtrate and reuse the collection tube.

13. Repeat Steps 10-12 until all of the cleared supernatant has been transferred to the HiBind® DNA Maxi Column.

14. Add 10 mL HBC Buffer.

    Note: HBC Buffer must be diluted with 100% isopropanol prior to use. Please see the instructions in the "Preparing Reagents" section on Page 6.

15. Centrifuge at 4,000*g* for 5 minutes.

16. Discard the filtrate and reuse the collection tube.

17. Add 15 mL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see the instructions in the "Preparing Reagents" section on Page 6.

18. Centrifuge at 4,000*g* for 5 minutes.

19. Discard the filtrate and reuse the collection tube.

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

21. Centrifuge the empty HiBind® DNA Maxi Column at 4,000*g* for 10 minutes to dry the column matrix.

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

22. Transfer the HiBind® DNA Maxi Column to a nuclease-free 50 mL centrifuge tube (not provided).

23. Add 1.5-3 mL Elution Buffer or sterile deionized water directly to the center of the column membrane.

24. Let it sit at room temperature for 5 minutes.

25. Centrifuge at 4,000*g* for 5 minutes.

    Note: This represents approximately 65-80% 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.

26. Store DNA at -20°C.

DNA Precipitation

The concentration of the eluted plasmid DNA varies with copy number, host strain, and growth conditions. In some cases, residual ethanol may also be present. To adjust the DNA concentration following plasmid DNA elution or for the removal of residual ethanol, perform the following isopropanol precipitation protocol.

1. Carefully transfer the eluted plasmid DNA to a clean tube suitable for precipitation. Add 1/10 volume 3M NaAC (pH 5.2) and 0.7 volume 100% isopropanol (room temperature). Vortex to mix.

2. Centrifuge at ≥15,000*g* for 20 minutes at 4ºC.

3. Carefully decant the supernatant.

4. Add 1-2 mL 70% ethanol. Vortex to resuspend the pellet.

5. Centrifuge at ≥15,000*g* for 10 minutes at 4ºC.

6. Carefully decant the supernatant.

7. Air dry the pellet for 10 minutes.

8. Add 200-500 µL Elution Buffer.

9. Store DNA at -20°C.

E.Z.N.A.® Plasmid DNA Midi/Maxi Kit Protocol - Low Copy Number Plasmid and Cosmid DNA Protocol

Low copy number plasmids generally give 0.1-1 µg DNA per mL overnight culture. For the isolation of low copy number plasmids, use the following modified protocol.

Note: The E.Z.N.A.® Plasmid DNA Midi Kit and the E.Z.N.A.® Plasmid DNA Maxi Kit come with enough buffers to perform the standard protocols. Additional buffers are needed to perform the Low Copy Number Plasmid and Cosmid DNA Protocol. These buffers can be purchased separately.

1. Increase the volume of starting culture from that of high copy number plasmids. Use 50-100 mL bacterial culture for the E.Z.N.A.® Plasmid DNA Midi Kit and 200-400 mL bacterial culture for the E.Z.N.A.® Plasmid DNA Maxi Kit.

2. Pellet the bacterial cells by centrifugation.

3. Decant or aspirate and discard the culture media.

4. Perform Steps 4-8 in the standard protocols with double volumes of Solution I, Solution II, and Solution III.

5. Continue with Step 9 of the standard protocols 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**.

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.
Bacterial culture is overgrown or not fresh	• Do not incubate cultures for more than 16 hours at 37ºC. • Storage of cultures for extended periods prior to plasmid isolation is detrimental.
Low elution efficiency	• pH of sterile deionized water must be ≥ 8.5.
Low copy number plasmid used	• Such plasmids may yield as little as 0.1 µg plasmid DNA from a 1 mL overnight culture. • Double culture volume and follow the Low Copy Number Plasmid and Cosmid DNA Protocol.
Columns were spun in a fixed angle rotor or with insufficient g-force	• For the Midi and Maxi kits, the columns must be spun in a swing bucket rotor at 4,000*g* for liquids to pass through efficiently.
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 OD. 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 according to instructions on Page 6.
HBC Buffer not diluted with 100% isopropanol	• Prepare HBC Buffer according to instructions on Page 6.
High molecular weight DNA contamination of product	
Cell lysate over mixed upon addition of Solution II	• Do not vortex or mix aggressively after adding Solution II.
Culture overgrown	• Overgrown culture contains lysed cells and degraded DNA. Do not grow cell for longer than 16 hours.

Troubleshooting Guide

RNA visible on agarose gel	
RNase A not added to Solution I	• Check that RNase A provided with the kit has been used. If Solution I is more than 6 months old, add more RNase A.
DNA floats out of well while loading agarose gel	
Ethanol has not been removed completely from column following wash steps	• Centrifuge column as instructed to dry the column before elution. • Incubate columns for 10 minutes at 65°C to completely dry membrane after centrifugation step.
Absorbance of purified DNA does not accurately reflect quality of the plasmid DNA (A_{260}/A_{280} ratio is too high or too low)	
DNA Wash Buffer is diluted with ethanol containing impurities	• Check the absorbance of the ethanol between 250 nm and 300 nm. Do not use ethanol with high absorbance. • Trace impurities may remain on the column after washing and can contribute to the absorbance.
Plasmid DNA is contaminated with RNA; RNase A treatment is insufficient.	• Confirm that the RNase A 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 fine silica particulates	• Spin the DNA sample at maximum speed for 1 minute; 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 neutralization.
4,000*g* centrifuge not available	
4,000*g* centrifuge not available	For centrifuges only capable of 2,000-4,000*g*, increase all centrifugation times by 2 minutes except for the drying of the column. Increase drying by 5 minutes. It may be necessary to incubate the empty column for drying step at 65°C for 10 minutes to completely dry the column. A swing bucket centrifuge is required.

For more purification solutions, visit **www.omegabiotek.com**

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