

E.Z.N.A.® FastFilter Plasmid DNA Midi Kit
E.Z.N.A.® FastFilter Plasmid DNA Maxi Kit

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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. The 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.® FastFilter 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 simultaneously processed. This system also includes a special filter cartridge which replaces the centrifugation step following alkaline lysis. Plasmid DNA purified by this system is suitable for automated fluorescent DNA sequencing, restriction endonuclease digestion, transfection of mammalian cells, and other manipulations.

Up to 150-200 µg high copy number plasmid DNA or 10-75 µg low copy number plasmid DNA can be purified from 15-50 mL overnight culture using the E.Z.N.A.® FastFilter Plasmid DNA Midi Kit. Yields vary according to plasmid copy number, *E. coli* strain, and growth conditions.

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 the E.Z.N.A.® FastFilter Plasmid DNA Maxi Kit. Yields vary according to plasmid copy number, *E. coli* strain, and growth conditions.

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- HB Buffer has been replaced by HBC Buffer.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.

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 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. The expected yield of 50 mL overnight cultures (LB medium) with the E.Z.N.A.® FastFilter Plasmid DNA Midi and Maxi Kits are indicated in the following table.

Sample yields from a 50 mL starting culture.

Plasmid	Replicon	Copy Number	Expected Yield
pUC vectors	pMB1	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

Vacuum Protocol



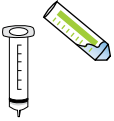
Pellet by Centrifugation



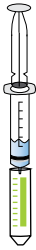
Resuspend
Lyse
Neutralize



Add GBT LysisBuffer



Transfer Lysate to
Lysate Clearance Syringe



Insert Plunger into Syringe
& Clear Lysate into
Centrifuge Tube



Add Equilibration Buffer
Pass Lysate through Column



Wash 3X



Transfer Column to Collection Tube
& Centrifuge to Dry HiBind® matrix



Transfer Column to new Centrifuge Tube
& Centrifuge to Elute DNA



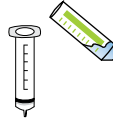
Pellet by Centrifugation



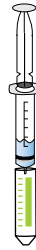
Resuspend
Lyse
Neutralize



Add GBT LysisBuffer



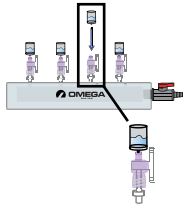
Transfer Lysate to
Lysate Clearance Syringe



Insert Plunger into Syringe
& Clear Lysate into
Centrifuge Tube



Prepare Vacuum Manifold



Insert Column into Luer Connector
on Vacuum Manifold

Add Equilibration Buffer

Pass Lysate through Column



Wash 3X



Transfer Column to Collection Tube
& Centrifuge to Dry HiBind® matrix



Transfer Column to new Centrifuge Tube
& Centrifuge to Elute DNA

Kit Contents

FastFilter Plasmid Midi Kit	D6905-00	D6905-01	D6905-03	D6905-04
Purifications	2	5	25	100
HiBind® DNA Midi Columns	2	5	25	100
15 mL Collection Tubes	2	5	25	100
Lysate Clearance Filter Syringe	2	5	25	100
Solution I	6 mL	30 mL	80 mL	270 mL
Solution II	6 mL	30 mL	80 mL	270 mL
N3 Buffer	4 mL	20 mL	40 mL	140 mL
GBT Buffer	6 mL	30 mL	60 mL	230 mL
HBC Buffer	5 mL	12 mL	58 mL	160 mL
DNA Wash Buffer	5 mL	15 mL	40 mL	4 x 40 mL
RNase A	20 µL	100 µL	300 µL	1.2 mL
Elution Buffer	5 mL	20 mL	60 mL	250 mL
User Manual	✓	✓	✓	✓

FastFilter Plasmid Maxi Kit	D6924-00	D6924-01	D6924-03	D6924-04
Purifications	2	5	25	100
HiBind® DNA Maxi Columns	2	5	25	100
50 mL Collection Tubes	2	5	25	100
Lysate Clearance Filter Syringe	2	5	25	100
Solution I	30 mL	60 mL	270 mL	4 x 270 mL
Solution II	30 mL	60 mL	270 mL	4 x 270 mL
N3 Buffer	15 mL	30 mL	135 mL	2 x 270 mL
GBT Buffer	20 mL	50 mL	230 mL	4 x 225 mL
HBC Buffer	18 mL	40 mL	190 mL	4 x 190 mL
DNA Wash Buffer	15 mL	40 mL	3 x 50 mL	12 x 50 mL
RNase A	120 µL	300 µL	1.2 mL	4 x 1.2 mL
Elution Buffer	15 mL	40 mL	150 mL	3 x 200 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
D6905-00	20 mL
D6905-01	60 mL
D6905-03	160 mL
D6905-04	160 mL per bottle
Kit	100% Ethanol to be Added
D6924-00	60 mL
D6924-01	160 mL
D6924-03	200 mL per bottle
D6924-04	200 mL per bottle

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

Kit	Isopropanol to be Added
D6905-00	2 mL
D6905-01	4.7 mL
D6905-03	23 mL
D6905-04	63 mL
Kit	Isopropanol to be Added
D6924-00	7 mL
D6924-01	16 mL
D6924-03	76 mL
D6924-04	76 mL per bottle

4. Check Solution II and GBT Buffer for precipitation before use. Redissolve any precipitation by warming to 37°C.

Storage and Stability

All of the E.Z.N.A.® FastFilter Plasmid DNA Midi Kit and FastFilter Plasmid DNA Maxi Kit 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. All other materials should be stored at room temperature. Solution II must be tightly capped when not in use.

Guidelines for Vacuum Manifold

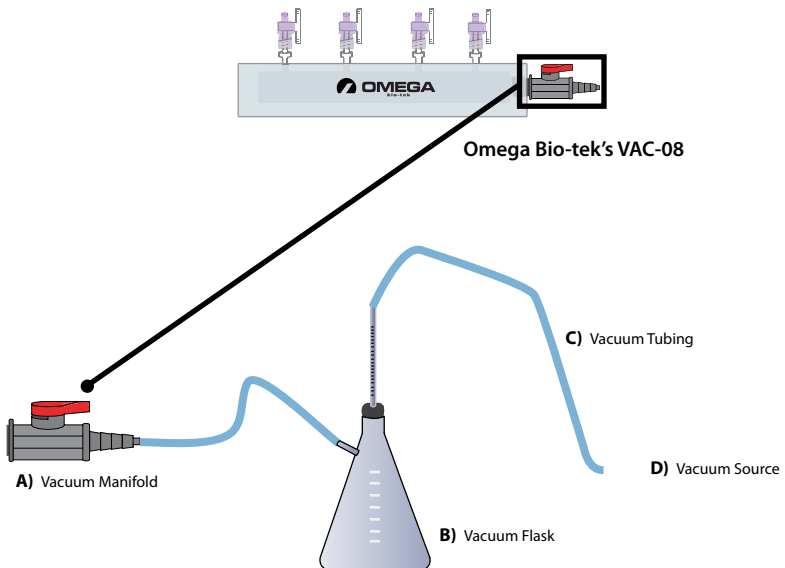
The following is required for use with the Vacuum/Spin Protocol:

- A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-08)
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma Aldrich VM20, 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
Torr (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Vacuum Setup:



Recommended Settings

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 DH5 α [™], DH1, and C600. These host strains yield high-quality DNA with E.Z.N.A.[®] FastFilter Plasmid DNA Midi and Maxi Kit 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 activity 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, N3 Buffer, and GBT Buffer, 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.[®] FastFilter Plasmid DNA Midi and Maxi 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 Midi and Maxi Columns.

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

Recommended Settings

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.

FastFilter Plasmid DNA Midi Kit Vacuum Protocol

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

All centrifugation steps should be performed with a swing bucket rotor for maximum plasmid DNA yields. All centrifugation steps should be carried out at room temperature. If high yields of low copy number plasmid DNA are desired, see “Low Copy Number Plasmids and Cosmids” on Page 27.

Materials and Equipment to be Supplied by User:

- 100% ethanol
- Isopropanol
- Centrifuge with a swing bucket rotor capable of at least 4,000 x g
- Vacuum Manifold
- Nuclease-free 15 mL and 50 mL centrifuge tubes
- Ice bucket
- Optional: Water bath or incubator capable of 70°C
- Optional: 3M NaOH

Before Starting:

- Chill N3 Buffer on ice
- Heat Elution Buffer to 70°C if plasmid DNA is >10 kb
- Prepare DNA Wash Buffer, HBC Buffer, and Solution I according to the instructions in the “Preparing Reagents” section on Page 6

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 ($OD_{600} \times \text{mL culture}$) 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® matrix 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 x g for 10 minutes at room temperature.

FastFilter Plasmid DNA Midi Kit Vacuum Protocol

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 2.5 mL 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 6.

5. Add 2.5 mL Solution II. Invert and rotate the tube gently 8-10 times to obtain a cleared lysate. This may require a 2 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 CO₂ in the air.

6. Add 1.25 mL cold N3 Buffer. Gently invert 10 times or until a flocculent white precipitate forms. This may require a 2 minute incubation at room temperature with occasional mixing.

Note: The solution must be mixed thoroughly. This is vital for obtaining good yields. If the mixture still appears viscous, brownish, or conglobated, more mixing is required to completely neutralize the solution.

7. Add 2 mL GBT Buffer. Gently invert the tube 4 times.

8. Prepare a Lysate Clearance Filter Syringe by removing the plunger. Place the barrel in a tube rack to keep upright. Make sure the end cap is attached to the syringe tip.

9. Immediately transfer the lysate from Step 7 into the barrel of the Lysate Clearance Filter Syringe.

10. Let sit at room temperature for 2 minutes. Any white precipitates should float to the top.

FastFilter Plasmid DNA Midi Kit Vacuum Protocol

11. Hold the Lysate Clearance Filter Syringe barrel over a 15 mL centrifuge tube (not provided) and remove the end cap from the syringe tip.
12. Gently insert the plunger into the barrel to expel the cleared lysate into the 15 mL centrifuge tube.

Note: Some of the lysate may remain in the flocculent precipitate. DO NOT force this residual lysate through the filter.

13. Prepare the vacuum manifold by following the manufacturer's instructions.
14. 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. Turn on the vacuum source to draw the NaOH through the column.
 3. Turn off the vacuum.
15. Transfer 3.5 mL cleared lysate from Step 12 by CAREFULLY aspirating 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.
 16. Turn on the vacuum source to draw the lysate through the column.
 17. Turn off the vacuum.
 18. Repeat Steps 15-17 until all of the cleared lysate has been transferred to the HiBind® DNA Midi Column.
 19. Add 3 mL HBC Buffer.

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

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

FastFilter Plasmid DNA Midi Kit Vacuum Protocol

21. Turn off the vacuum.

22. 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.

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

24. Turn off the vacuum.

25. Repeat Steps 22-24 for a second DNA Wash Buffer wash step.

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

27. Centrifuge the empty HiBind® DNA Midi Column at 4,000 x 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.

28. Transfer the HiBind® DNA Midi Column to a new nuclease-free 15 mL centrifuge tube.

29. Add 0.5-1 mL Elution Buffer directly to the center of the column matrix.

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

31. Centrifuge at 4,000 x 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.

32. Store DNA at -20°C.

FastFilter Plasmid DNA Midi Kit Centrifugation Protocol

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

The following protocol is designed for plasmid DNA isolation when a vacuum manifold is not available. All centrifugation steps should be performed with a swing bucket rotor for maximum plasmid DNA yields. All centrifugation steps should be carried out at room temperature. If high yields of low copy number plasmid are desired, see “Low Copy Number Plasmids and Cosmids” on Page 27.

Materials and Equipment to be Supplied by User:

- 100% ethanol
- Isopropanol
- Centrifuge with a swing bucket rotor capable of at least 4,000 x g
- Nuclease-free 15 mL and 50 mL centrifuge tubes
- Ice bucket
- Optional: Water bath or incubator capable of 70°C
- Optional: 3M NaOH

Before Starting:

- Chill N3 Buffer on ice
- Heat Elution Buffer to 70°C if plasmid DNA is >10 kb
- Prepare DNA Wash Buffer, HBC Buffer, and Solution I according to the instructions in the “Preparing Reagents” section on Page 6

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 ($OD_{600} \times \text{mL culture}$) 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® matrix 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 x g for 10 minutes at room temperature.

FastFilter Plasmid DNA Midi Kit Centrifugation Protocol

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 2.5 mL 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 6.

5. Add 2.5 mL Solution II. Invert and rotate the tube gently 10 times to obtain a cleared lysate. This may require a 2 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 CO₂ in the air.

6. Add 1.25 mL cold N3 Buffer. Gently invert 10 times or until a flocculent white precipitate forms. This may require a 2 minute incubation at room temperature with occasional mixing.

Note: The solution must be mixed thoroughly. This is vital for obtaining good yields. If the mixture still appears viscous, brownish, or conglobated, more mixing is required to completely neutralize the solution.

7. Add 2 mL GBT Buffer. Gently invert the tube 4 times.

8. Prepare a Lysate Clearance Filter Syringe by removing the plunger. Place the barrel in a tube rack to keep upright. Make sure the end cap is attached to the syringe tip.

9. Immediately transfer the lysate from Step 7 into the barrel of the Lysate Clearance Filter Syringe.

10. Let sit at room temperature for 2 minutes. Any white precipitates should float to the top.

FastFilter Plasmid DNA Midi Kit Centrifugation Protocol

11. Hold the Lysate Clearance Filter Syringe barrel over a 15 mL centrifuge tube (not provided) and remove the end cap from the syringe tip.
12. Gently insert the plunger into the barrel to expel the cleared lysate into the 15 mL centrifuge tube.

Note: Some of the lysate may remain in the flocculent precipitate. DO NOT force this residual lysate through the filter.

Note: Steps 13-34 should be performed in a swing bucket rotor for maximum plasmid DNA yield. All of centrifugation steps should be carried out at room temperature.

13. Insert a HiBind® DNA Midi Column into a 15 mL Collection Tube (provided).

Optional Protocol for Column Equilibration:

1. Add 1 mL 3M NaOH to the HiBind® DNA Midi Column.
 2. Let sit at room temperature for 4 minutes.
 3. Centrifuge at 4,000 x *g* for 3 minutes.
 4. Discard the filtrate and reuse the collection tube.
-
14. Transfer 3.5 mL cleared lysate from Step 12 by CAREFULLY aspirating 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.
 15. Centrifuge at 4,000 x *g* for 3 minutes.
 16. Discard the filtrate and reuse the collection tube.
 17. Repeat Steps 14-16 until all of the cleared lysate has been transferred to the HiBind® DNA Midi Column.
 18. Add 3 mL HBC Buffer.

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

FastFilter Plasmid DNA Midi Kit Centrifugation Protocol

19. Centrifuge at 4,000 x *g* for 3 minutes.

20. Discard the filtrate and reuse the collection tube.

21. 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.

22. Centrifuge at 4,000 x *g* for 3 minutes.

23. Discard the filtrate and reuse the collection tube.

24. Repeat Steps 21-23 for a second DNA Wash Buffer wash step.

25. Centrifuge the empty HiBind® DNA Midi Column at 4,000 x *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.

26. Transfer the HiBind® DNA Midi Column to a new nuclease-free 15 mL centrifuge tube.

27. Add 0.5-1 mL Elution Buffer directly to the center of the column matrix.

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

29. Centrifuge at 4,000 x *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.

30. Store DNA at -20°C.

FastFilter Plasmid DNA Maxi Kit Vacuum Protocol

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

All centrifugation steps should be performed with a swing bucket rotor for maximal plasmid DNA yields. All centrifugation steps should be carried out at room temperature. If high yields of low copy number plasmid DNA are desired, see “Low Copy Number Plasmids and Cosmids” on Page 27.

Materials and Equipment to be Supplied by User:

- 100% ethanol
- Isopropanol
- Centrifuge with a swing bucket rotor capable of at least 4,000 x g
- Vacuum Manifold
- Nuclease-free 50 mL centrifuge tubes
- Appropriate centrifuge bottle for pelleting bacterial culture in Step 1
- Ice bucket
- Optional: Water bath or incubator capable of 70°C
- Optional: 3M NaOH

Before Starting:

- Chill N3 Buffer on ice
- Heat Elution Buffer to 70°C if plasmid DNA is >10 kb
- Prepare DNA Wash Buffer, HBC Buffer, and Solution I according to the instructions in the “Preparing Reagents” section on Page 6

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 ($OD_{600} \times \text{mL culture}$) 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® matrix 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 x g for 10 minutes at room temperature.

FastFilter Plasmid DNA Maxi Kit Vacuum Protocol

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 10 mL 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 6.

5. Add 10 mL Solution II. Invert and rotate the tube gently 10 times to obtain a cleared lysate. This may require a 2 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 CO₂ in the air.

6. Add 5 mL cold N3 Buffer. Gently invert 10 times or until a flocculent white precipitate forms. This may require a 2 minute incubation at room temperature with occasional mixing.

Note: The solution must be mixed thoroughly. This is vital for obtaining good yields. If the mixture still appears viscous, brownish, or conglobated, more mixing is required to completely neutralize the solution.

7. Add 8.3 mL GBT Buffer. Gently invert the tube 4 times.

8. Prepare a Lysate Clearance Filter Syringe by removing the plunger. Place the barrel in a tube rack to keep upright. Make sure the end cap is attached to the syringe tip.

9. Immediately transfer the lysate from Step 7 into the barrel of the Lysate Clearance Filter Syringe.

10. Let sit at room temperature for 2 minutes. Any white precipitates should float to the top.

FastFilter Plasmid DNA Maxi Kit Vacuum Protocol

11. Hold the Lysate Clearance Filter Syringe barrel over a 50 mL centrifuge tube (not provided) and remove the end cap from the syringe tip.
12. Gently insert the plunger into the barrel to expel the cleared lysate into the 50 mL centrifuge tube.

Note: Some of the lysate may remain in the flocculent precipitate. DO NOT force this residual lysate through the filter.

13. Prepare the vacuum manifold by following the manufacturer's instructions.
14. 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. Turn on the vacuum source to draw the NaOH through the column.
 3. Turn off the vacuum.
15. Transfer 15 mL cleared lysate from Step 12 by CAREFULLY aspirating 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.
 16. Turn on the vacuum source to draw the lysate through the column.
 17. Turn off the vacuum.
 18. Repeat Steps 15-17 until all of the cleared lysate has been transferred to the HiBind® DNA Maxi Column.

FastFilter Plasmid DNA Maxi Kit Vacuum Protocol

19. Add 10 mL HBC Buffer.

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

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

21. Turn off the vacuum.

22. 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.

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

24. Turn off the vacuum.

25. Add 10 mL DNA Wash Buffer.

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

27. Turn off the vacuum.

28. Transfer the HiBind® DNA Maxi Column to a 50 mL Collection Tube (provided).

29. Centrifuge the empty HiBind® DNA Maxi Column at 4,000 x 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.

FastFilter Plasmid DNA Maxi Kit Vacuum Protocol

30. Transfer the HiBind® DNA Maxi Column to a nuclease-free 50 mL centrifuge tube.
31. Add 1.5-3 mL Elution Buffer directly to the center of the column matrix.
32. Let it sit at room temperature for 3 minutes.
33. Centrifuge at 4,000 x *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.

34. Store DNA at -20°C.

FastFilter Plasmid DNA Maxi Kit Centrifugation Protocol

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

The following protocol is designed for plasmid DNA isolation when a vacuum manifold is not available. All centrifugation steps should be performed with a swing bucket rotor for maximum plasmid DNA yields. All centrifugation steps should be carried out at room temperature. If high yields of low copy number plasmid are desired, see “Low Copy Number Plasmids and Cosmids” on Page 27.

Materials and Equipment to be Supplied by User:

- 100% ethanol
- Isopropanol
- Centrifuge with a swing bucket rotor capable of at least 4,000 x g
- Appropriate centrifuge bottle for pelleting bacterial culture in Step 1
- Nuclease-free 50 mL centrifuge tubes
- Ice bucket
- Optional: Water bath or incubator capable of 70°C
- Optional: 3M NaOH

Before Starting:

- Chill N3 Buffer on ice
- Heat Elution Buffer to 70°C if plasmid DNA is >10 kb
- Prepare DNA Wash Buffer, HBC Buffer, and Solution I according to the instructions in the “Preparing Reagents” section on Page 6

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 ($OD_{600} \times \text{mL culture}$) 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® matrix 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 x g for 10 minutes at room temperature.

FastFilter Plasmid DNA Maxi Kit Centrifugation Protocol

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 10 mL 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 6.

5. Add 10 mL Solution II. Invert and rotate the tube gently 10 times to obtain a cleared lysate. This may require a 2 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 CO₂ in the air.

6. Add 5 mL cold N3 Buffer. Gently invert 10 times or until a flocculent white precipitate forms. This may require a 2 minute incubation at room temperature with occasional mixing.

Note: The solution must be mixed thoroughly. This is vital for obtaining good yields. If the mixture still appears viscous, brownish, or conglobated, more mixing is required to completely neutralize the solution.

7. Add 8.3 mL GBT Buffer. Gently invert the tube 4 times.

8. Prepare a Lysate Clearance Filter Syringe by removing the plunger. Place the barrel in a tube rack to keep upright. Make sure the end cap is attached to the syringe tip.

9. Immediately transfer the lysate from Step 7 into the barrel of the Lysate Clearance Filter Syringe.

10. Let sit at room temperature for 2 minutes. Any white precipitates should float to the top.

FastFilter Plasmid DNA Maxi Kit Centrifugation Protocol

11. Hold the Lysate Clearance Filter Syringe barrel over a 50 mL centrifuge tube (not provided) and remove the end cap from the syringe tip.
12. Gently insert the plunger into the barrel to expel the cleared lysate into the 50 mL centrifuge tube.

Note: Some of the lysate may remain in the flocculent precipitate. DO NOT force this residual lysate through the filter.

Note: Steps 13-36 should be performed in a swing bucket rotor for maximum plasmid DNA yield. All of centrifugation steps should be carried out at room temperature.

13. 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 x *g* for 3 minutes.
 4. Discard the filtrate and reuse the collection tube.
-
14. Transfer 15 mL cleared lysate from Step 12 by CAREFULLY aspirating 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.
 15. Centrifuge at 4,000 x *g* for 3 minutes.
 16. Discard the filtrate and reuse the collection tube.
 17. Repeat Steps 14-16 until all of the cleared lysate has been transferred to the HiBind® DNA Maxi Column.
 18. Add 10 mL HBC Buffer.

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

FastFilter Plasmid DNA Maxi Kit Centrifugation Protocol

19. Centrifuge at 4,000 x *g* for 3 minutes.

20. Discard the filtrate and reuse the collection tube.

21. 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.

22. Centrifuge at 4,000 x *g* for 3 minutes.

23. Discard the filtrate and reuse the collection tube.

24. Add 10 mL DNA Wash Buffer.

25. Centrifuge at 4,000 x *g* for 3 minutes.

26. Discard the filtrate and reuse the collection tube.

27. Centrifuge the empty HiBind® DNA Maxi Column at 4,000 x *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.

FastFilter Plasmid DNA Maxi Kit Centrifugation Protocol

28. Transfer the HiBind® DNA Maxi Column to a nuclease-free 50 mL centrifuge tube.

29. Add 1.5-3 mL Elution Buffer directly to the center of the column matrix.

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

31. Centrifuge at 4,000 x *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.

32. 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 isopropanol (room temperature). Vortex to mix.
2. Centrifuge at $\geq 15,000 \times g$ for 20 minutes at 4°C.
3. Carefully decant the supernatant.
4. Add 1-2 mL 70% ethanol. Vortex to resuspend the pellet.
5. Centrifuge at $\geq 15,000 \times g$ for 10 minutes at 4°C.
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.

Low Copy Number Plasmid and Cosmid DNA Purification

E.Z.N.A.[®] FastFilter 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 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.[®] FastFilter Plasmid DNA Midi Kit and the E.Z.N.A.[®] FastFilter 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. See Page 31 for ordering information.

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.[®] FastFilter Plasmid DNA Midi Kit and 200-400 mL bacterial culture for the E.Z.N.A.[®] FastFilter Plasmid DNA Maxi Kit.
2. Pellet the bacterial cells by centrifugation.
3. Decant or aspirate and discard the culture media.
4. Perform Steps 4-7 in the standard protocols with double volumes of Solution I, Solution II, N3 Buffer, and GBT Buffer.
5. Continue with Step 8 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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Do not incubate cultures for more than 16 hours at 37°C. • Storage of cultures for extended periods prior to plasmid isolation is detrimental.
Low elution efficiency	<ul style="list-style-type: none"> • If using endotoxin-free water for elution, the pH must be 8.0
Low copy number plasmid used	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • For the Midi and Maxi kits, the columns must be spun in a swing bucket rotor at 4,000 x g for liquids to pass through efficiently.
Alkaline lysis is prolonged	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Prepare DNA Wash Buffer according to the instructions on Page 6.
HBC Buffer not diluted with isopropanol	<ul style="list-style-type: none"> • Prepare HBC Buffer according to the instructions on Page 6.

Troubleshooting Guide

High molecular weight DNA contamination of product	
Over mixing of cell lysate upon addition of Solution II	<ul style="list-style-type: none"> Do not vortex or mix aggressively after adding Solution II.
Culture overgrown	<ul style="list-style-type: none"> 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 been removed completely from column following wash steps	<ul style="list-style-type: none"> Centrifuge column as instructed to dry the column before elution. Incubate columns for 10 minutes at 65°C to completely dry matrix after centrifugation step.
Absorbance of purified DNA does not accurately reflect quality of the plasmid (A_{260}/A_{280} ratio is too high or too low)	
DNA Wash Buffer is diluted with ethanol containing impurities	<ul style="list-style-type: none"> 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.	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> Reduce the initial volume of culture.
Plasmid DNA is contaminated with chromosomal DNA	<ul style="list-style-type: none"> 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 after adding N3 Buffer.
4,000 x g centrifuge not available	
4,000 x g centrifuge not available	<p>For centrifuges only capable of 2,000-4,000 x 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.</p> <p><i>A Swing Bucket Centrifuge is Required.</i></p>

Ordering Information

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

Product	Part Number
Solution I (250 mL)	PS001
Solution II (250 mL)	PS002
N3 Buffer (135 mL)	PS095
Elution Buffer (100 mL)	PDR048
GBT Buffer (100 mL)	PS015
DNA Wash Buffer (100 mL)	PS010
RNase A (5 mL)	AC117

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