omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® FastFilter Plasmid DNA Mini Kit

D6944-00	10 preps
D6944-01	100 preps
D6944-02	300 preps

Manual Date: April 2024
Manual Revision: v3.1

For Research Use Only

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E.Z.N.A.® FastFilter Plasmid DNA Mini Kit

Table of Contents

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Intended Use

For professional research use.

The E.Z.N.A.® FastFilter Plasmid DNA Mini Kit is intended for isolation and purification of plasmid DNA from up to 5 mL bacterial culture for high copy number plasmids and up to 10 mL bacterial culture for low copy number plasmids. Please ensure that the cell density does not exceed an OD_{600} of 2.0.

Intended User

The E.Z.N.A.® FastFilter Plasmid DNA Mini Kit is intended for professional use and to be used by or under the supervision of professional users, such as laboratory personnel, technicians, researchers and physicians specifically instructed and trained in molecular biology techniques.

Introduction

The E.Z.N.A.® FastFilter Plasmid DNA Mini Kit is designed for rapid purification of high-quality plasmid DNA from up to 5 mL of culture following alkaline-lysis method. The kit features an innovative and first of its kind FastFilter Mini Column that is nested within a regular HiBind® DNA Mini Column to combine lysate clearance and DNA binding into one or two centrifugation steps depending on the culture volume. The protocol time is just 9 minutes when starting with bacterial pellet from up to 3 mL culture volume. The protocol time for culture volumes greater than 3 mL will be slightly longer due to two centrifugation steps for lysate clearance. Following wash and elute steps, purified plasmid DNA is immediately ready for a wide variety of downstream applications such as routine screening, restriction enzyme digestion, transformation, PCR, and DNA sequencing.

Typically, up to 5 mL overnight bacterial culture in LB or other suitable growth medium is centrifuged to pellet the bacterial cells. The cells are then lysed under alkaline conditions and subsequently neutralized to form white flocculent precipitate. Neutralized lysate is then transferred to a FastFilter Mini Column nested within a HiBind® DNA Mini Column. Upon adjustment of binding conditions, the lysate is centrifuged through this FastFilter-HiBind® configuration to both clear the lysate and facilitate binding of DNA to the HiBind® DNA Mini Column. The FastFilter Mini Column is discarded, and the HiBind® DNA Mini Column is subjected to two wash steps to remove salt and other contaminants. High-quality plasmid DNA is then eluted in low-salt elution buffer and is ready for use in wide range of downstream applications mentioned above.

New in this Edition:

April 2024

- Addition of Warnings and Safety Information.

Kit Contents

Product	D6944-00	D6944-01	D6944-02
Purifications	10	100	300
FastFilter Mini Columns	10	100	300
HiBind® DNA Mini Columns	10	100	300
2 mL Collection Tubes	10	100	300
Solution I	3 mL	20 mL	60 mL
Solution II	3 mL	20 mL	60 mL
N3 Buffer	2.5 mL	10 mL	35 mL
GBT Buffer	6 mL	20 mL	2 x 20 mL
HBC Buffer	5 mL	25 mL	80 mL
DNA Wash Buffer	2.5 mL	25 mL	2 x 25 mL
Elution Buffer	2 mL	15 mL	30 mL
RNase A	Pre-Added	100 µL	400 µL
User Manual	✓	✓	✓

Storage and Stability

All of the E.Z.N.A.® FastFilter Plasmid DNA Mini Kit components are guaranteed for at least 12 months from date of purchase when stored as follows. RNase A and Solution I (once RNase A is added) should be stored at 2-8˚C. Store all other components at room temperature and away from bright light. Solution II must be tightly capped when not in use. During shipment or storage in cool ambient conditions, precipitates may form in some buffers. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Warnings

This kit is for research use only.

Please read all instructions carefully before using the kit.

Decontaminate and dispose of all potentially infectious materials in accordance with applicable local, state, and national regulations. Please refer to safety data sheets (SDSs) for information on disposal of different components included in this kit.

Safety Information

All chemicals and biological materials are potentially hazardous. Biological samples such as plasma, serum, tissues, body fluids, blood etc. are potentially infectious and must be treated as biohazardous materials. Conduct all work in properly equipped facilities following universal precautions and using appropriate personal safety equipment such as disposable gloves, lab coats, safety glasses etc. as required by policies and procedures outlined by your facility. Please refer to safety data sheets (SDSs) for information on safe handling, transport and disposal of different components included in this kit. SDSs are made available in PDF format on the product page at www.omegabiotek.com. Discard all waste in accordance with the local safety regulations.

Some of the buffers included in the product contain guanidine-based chaotropic agents, which can form highly reactive compounds when combined with bleach. DO NOT add bleach or acidic solutions to guanidine-containing waste. Please access the SDSs online for detailed information on the reagents.

Preparing Reagents

1. Add the vial of RNase A to the bottle of Solution I and store at 2-8˚C (100 and 300 Purification sizes only).

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

Kit	100% Isopropanol to be Added
D6944-00	2 mL
D6944-01	10 mL
D6944-02	32 mL

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

Kit	100% Ethanol to be Added
D6944-00	10 mL
D6944-01	100 mL
D6944-02	100 mL per bottle

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

Precautions

Some of the buffers included in the E.Z.N.A® Fastfilter Plasmid DNA Mini Kit contain guanidine-based chaotropic agents, which can form highly reactive compounds when combined with bleach. **DO NOT add bleach or acidic solutions** to guanidine containing sample-preparation waste. Please access the SDSs online for detailed information on the reagents.

Component	Description
RNase A 	Contains: Ribonuclease A. Danger! Causes an allergic skin irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust/fume/gas/mist/vapors/spray. Wear protective gloves/protective clothing/eye protection/face protection. Wear respiratory protection in case of inadequate ventilation. Contaminated work clothing must not be allowed out of the workplace. INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Call a poison center/doctor/physician/first aider if experience respiratory symptoms. ON SKIN: Wash with plenty of water. Get medical attention if skin irritation or rash occurs. Take off contaminated clothing and wash it before reuse.
Solution I with RNase A 	Contains: Ribonuclease A. Danger! Causes an allergic skin irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust/fume/gas/mist/vapors/spray. Wear protective gloves/protective clothing/eye protection/face protection. Wear respiratory protection in case of inadequate ventilation. Contaminated work clothing must not be allowed out of the workplace. INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Call a poison center/doctor/physician/first aider if experience respiratory symptoms. ON SKIN: Wash with plenty of water. Get medical attention if skin irritation or rash occurs. Take off contaminated clothing and wash it before reuse.
Solution II 	Contains: Sodium hydroxide and anionic detergent. Warning! Causes serious eye irritation. Wear protective gloves/protective clothing/eye protection/face protection. IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/attention if eye irritation persists.

Precautions

Component	Description
GBT Buffer 	Contains: Guanidine hydrochloride. Warning! Causes serious eye irritation. Harmful if swallowed. Causes skin irritation. Do not eat, drink or smoke when using this product. Wear protective gloves/ protective clothing/eye protection/face protection. IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/attention if eye irritation persists. Take off contaminated clothing and wash before resuse. ON SKIN: Wash with plenty of water and soap. Get medical advice/attention if skin irritation occurs. SWALLOWED: Rinse mouth. Call a poison center or doctor/ physician if you feel unwell.
N3 Buffer 	Contains: Acetic acid glacial. Danger! Causes severe skin burns and eye damage. Do not breathe mist/vapors/spray. Wear protective gloves/protective clothing/eye protection/face protection. IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Wash contaminated clothing before reuse. ON SKIN (or hair): Remove/ Take off immediately all contaminated clothing. Rinse skin with water/shower. SWALLOWED: Rinse mouth. Do NOT induce vomiting. Immediately call a poison center or doctor/physician. INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
HBC Buffer 	Contains: Guanidine hydrochloride. Warning! Causes serious eye irritation. Harmful if swallowed. Causes skin irritation. Do not eat, drink or smoke when using this product. Wear protective gloves/ protective clothing/eye protection/face protection. IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/attention if eye irritation persists. Take off contaminated clothing and wash before resuse. ON SKIN: Wash with plenty of water and soap. Get medical advice/attention if skin irritation occurs. SWALLOWED: Rinse mouth. Call a poison center or doctor/ physician if you feel unwell.

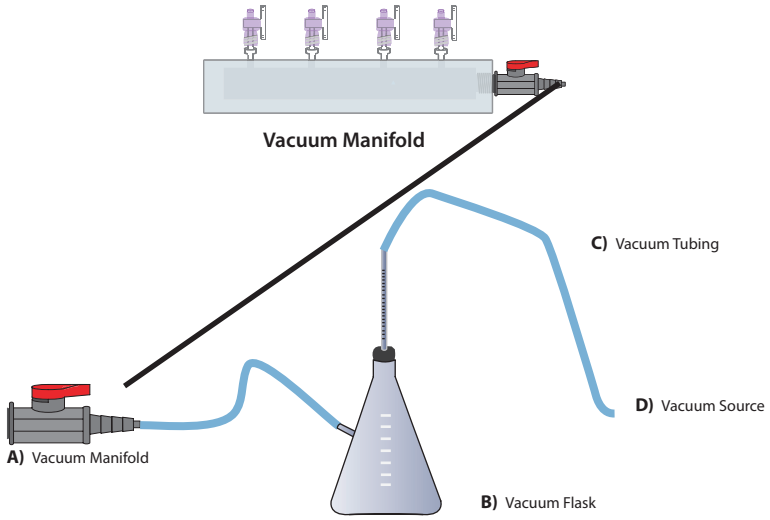
Guidelines for Vacuum Manifold

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

A) Vacuum Manifold
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 (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:

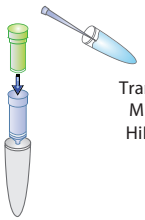


Spin Protocol

Pellet by Centrifugation

Resuspend and Lyse

Neutralize



Transfer Lysate to FastFilter Mini Column, nested in a HiBind® DNA Mini Column

Clear Lysate and Bind

Wash 2X

Dry

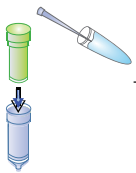
Elute

Vacuum/Spin Protocol

Pellet by Centrifugation

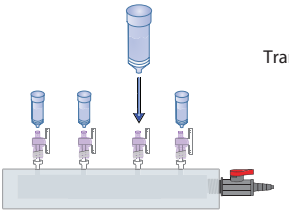
Resuspend and Lyse

Neutralize



Transfer Lysate to FastFilter Mini Column, nested in a HiBind® DNA Mini Column

Clear Lysate and Bind



Transfer to manifold

Wash 2X

Dry

Elute

Growth and Culture of Bacteria

Bacterial Strain Selection

It is strongly recommended that an EndA negative strain of *E. coli* be used for routine plasmid isolation. Examples of such strains include DH5α™ , DH1, and C600. 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 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 of the indicated volume of the container.

Culture Media

The E.Z.N.A.® FastFilter Plasmid DNA Mini Kit is specifically designed for use with cultures grown in Luria Bertani (LB) medium up to 5 mL. Richer broths such as TB (Terrific Broth) or 2xYT lead to high cell densities that can overload the purification system. In such a scenario, recommended culture volumes must be reduced to not exceed the capacity of the FastFilter and HiBind® DNA Mini Column configuration as described in the section below.

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

To ensure proper purification, the starting culture volume should be based on culture cell density. An optical density between 1.0 and 2.0 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 2.0. Using a high-density culture outside of the recommended OD range may overload the purification system. In such a scenario, culture volume may need to be reduced to not exceed the capacity of the FastFilter and HiBind® DNA Mini Column configuration.

Centrifugation Protocol - Up to 3 mL Culture Volume

This protocol is designed to isolate plasmid DNA from *E. coli* grown in an overnight culture up to 3 mL LB medium.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000*g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Culture tubes
- 100% ethanol
- 100% isopropanol
- Optional: sterile deionized water
- Optional: water bath or incubator capable of 70°C

Before Starting:

- Prepare DNA Wash Buffer, HBC Buffer, and Solution I according to the instructions in the "Preparing Reagents" section on Page 6.

1. Isolate a single colony from a freshly streaked selective plate, and inoculate a culture of up to 3 mL LB or suitable growth medium containing the appropriate selective antibiotic. Incubate for ~12-16 hours at 37°C with vigorous shaking (~ 300 rpm). Use a 10-15 mL culture tube or a flask with a volume of at least 4 times the volume of the culture. It is strongly recommended that an EndA negative strain of *E. coli* be used for routine plasmid isolation. Examples of such strains include DH5α® and JM109®. Transfer to a conical vial (not included) after incubation.

2. Centrifuge at 13,000*g* for 1 minute at room temperature.

3. Decant or aspirate and discard the culture media.

4. Add 175 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 6.

5. Add 175 µL 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.

6. Add 85 µL N3 Buffer. Gently invert 10 times or until a flocculent white precipitate forms.

   Note: Solution must be mixed thoroughly and immediately after adding N3 Buffer to avoid localized precipitation.

7. Add 100 µL GBT Buffer. Immediately invert 10 times to mix.

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

9. Insert a FastFilter Mini Column into the HiBind® DNA Mini Column.

10. Transfer the lysate from Step 7 by pipetting or pouring into the FastFilter Mini Column.

11. Centrifuge at 13,000*g* for 1 minute.

12. Remove and discard the FastFilter Mini Column only.

13. Discard the filtrate from the collection tube and attach it back to HiBind DNA Mini Column used in Step 8.

14. Add 200 µL HBC Buffer to the HiBind® DNA Mini Column.

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

15. Centrifuge at 13,000*g* for 10 seconds.

    Note: It is not necessary to discard supernatant after centrifugation. This will not interfere with the next step.

16. Add 400 µL DNA Wash Buffer.

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

17. Centrifuge at 13,000*g* for 2 minutes to dry column.

    Note: Carefully remove the column after centrifugation without disturbing the flow through. Residual ethanol may interfere with downstream applications. If column is wet, discard the filtrate and reuse the collection tube. Centrifuge again at 13,000*g* for 2 minutes.

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

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

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

    Note: For purification of plasmid DNA >10 kb, using preheated Elution Buffer at 70°C may boost yields.

20. Centrifuge at 13,000*g* for 1 minute.

    Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

21. Store plasmid DNA at -20°C.

Vacuum Protocol - Up to 3 mL Culture Volume

This protocol is designed to isolate plasmid DNA from *E. coli* grown in an overnight culture up to 3 mL LB medium. See page 9 for guidelines on preparing the vacuum manifold used in this protocol.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000*g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Culture tubes
- 100% ethanol
- 100% isopropanol
- Optional: sterile deionized water
- Optional: water bath or incubator capable of 70°C

Before Starting:

- Prepare DNA Wash Buffer, HBC Buffer, and Solution I according to the instructions in the "Preparing Reagents" section on Page 6.

1. Isolate a single colony from a freshly streaked selective plate, and inoculate a culture of up to 3 mL LB or suitable growth medium containing the appropriate selective antibiotic. Incubate for ~12-16 hours at 37°C with vigorous shaking (~ 300 rpm). Use a 5-15 mL culture tube or a flask with a volume of at least 4 times the volume of the culture. It is strongly recommended that an EndA negative strain of *E. coli* be used for routine plasmid isolation. Examples of such strains include DH5α® and JM109®. Transfer to a conical vial (not included) after incubation.

2. Centrifuge at 13,000*g* for 1 minute at room temperature.

3. Decant or aspirate and discard the culture media.

4. Add 175 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 6.

5. Add 175 µL 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.

6. Add 85 µL N3 Buffer. Gently invert 10 times or until a flocculent white precipitate forms.

   Note: Solution must be mixed thoroughly and immediately after adding N3 Buffer to avoid localized precipitation

7. Add 100 µL GBT Buffer. Immediately invert 10 times to mix.

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

9. Insert a FastFilter Mini Column into the HiBind® DNA Mini Column.

10. Transfer the lysate from Step 7 by pipetting or pouring into the FastFilter Mini Column.

11. Centrifuge at 13,000*g* for 1 minute.

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

13. Remove and discard the FastFilter Mini Column only. Discard the filtrate and save the 2 mL Collection Tube for later use in the protocol.

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

15. Add 200 µL HBC.

    Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see 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 400 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see 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. Transfer the HiBind® DNA Mini Column to the 2 mL Collection Tube saved from Step 13.

22. Centrifuge the empty HiBind® DNA Mini Column at 13,000*g* for 2 minutes 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.

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

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

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

    Note: For purification of plasmid DNA >10 kb, using preheated Elution Buffer at 70°C may boost yields.

25. Centrifuge at 13,000*g* for 1 minute.

    Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

26. Store plasmid DNA at -20°C.

Protocol - 3 to 5 mL Culture Volume or Low Copy Number Plasmid Purification

Low copy number plasmids generally give 0.1-1 µg DNA per mL overnight culture. For culture volumes between 3-5 mL or for isolation of low copy number plasmids, use the following modified protocol.

Important: The E.Z.N.A.® FastFilter Plasmid DNA Mini Kit comes with enough reagents to perform the standard protocols. Additional Solution I, Solution II, N3 Buffer, and GBT Buffer are needed to perform this modified protocol. Please visit the product page at **www.omegabiotek.com** or contact your Omega Bio-tek representative for more details and ordering information.

1. Bacterial culture volumes of 3-5 mL for high copy number plasmids and up to 10 mL of bacterial culture at OD_{600} of 2.0 for low copy number plasmids can be utilized in this protocol.

   Note: For high density cultures, the volume may need to be reduced to not exceed the capacity of the FastFilter and HiBind® DNA Mini Column Configuration.

2. Pellet the bacterial cells by centrifugation.

3. Decant or aspirate and discard the culture media.

4. Perform Steps 4-11 in the protocol on Pages 13 & 14 (Centrifugation Protocol - Up to 3 mL Culture Volume) or Pages 16 & 17 (Vacuum Protocol - Up to 3 mL Culture Volume) with double the volumes of **Solution I, Solution II, N3 Buffer, and GBT Buffer.**

5. Divide the lysate and pass it through the FastFilter and HiBind® DNA Mini Column configuration twice and ensure all the lysate has been transferred to the HiBind DNA Mini Column.

6. Continue with Step 12 of the protocol on Page 14 (Centrifugation Protocol - Up to 3 mL Culture Volume) or Page 17 (Vacuum Protocol - Up to 3 mL Culture Volume) 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
No/Low DNA Yields	DNA Wash Buffer not diluted with ethanol	Prepare DNA Wash Buffer according to instructions on Page 6.
	HBC Buffer not diluted with isopropanol	Prepare HBC Buffer according to instructions on Page 6.
	Poor cell lysis	Only use LB or YT medium containing antibiotic. Do not use more than 5 mL (high copy number plasmids) or 10 mL (low copy number plasmids) culture at a cell density of 2.0 at OD_{600}.
		Cells may not have been dispersed adequately prior to the addition of Solution II. Vortex to completely resuspend the cells.
		Increase Solution II incubation time to obtain a clear lysate.
		Solution II, if not tightly closed, may need to be replaced.
	Culture is overgrown or not fresh	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	The pH of Elution Buffer or water must be pH 8.0-9.0.
	Column matrix lost binding capacity during storage	Add 100 µL 3M NaOH to the HiBind® DNA Mini column prior to loading the sample. Centrifuge at 10,000*g* for 30 seconds. Discard the filtrate.
	FastFilter Mini Column capacity exceeded	Ensure sample input does not exceed 5 mL (high copy number plasmids) or 10 mL (low copy number plasmids) at an OD_{600} of 2.0. For 3-5 mL culture volumes and low copy number plasmid purification, follow the modified protocol on Page 20.
	Large-sized plasmid purification	Pre-heat the elution buffer to 70°C, incubate the elution buffer on the column for 5 minutes before eluting the plasmid DNA. A second elution step using the eluate from the first round may boost yields without decreasing the concentration of the plasmid DNA.

Problem	Cause	Solution
High molecular weight DNA contamination of product	Over mixing of cell lysate upon addition of Solution II	Do not vortex or mix aggressively after adding Solution II.
	Culture overgrown	Overgrown cultures contain lysed cells and degraded DNA. Do not grow the bacterial cultures for longer than 16 hours.
Problem	**Cause**	**Solution**
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.
Problem	**Cause**	**Solution**
Particulate passes through the FastFilter Mini Column into the HiBind® column or collection tube	Culture exceeds the maximum capacity of the FastFilter Mini Column	Reduce culture input. See Recommended Settings on page 11.

Problem	Cause	Solution
Absorbance of purified DNA does not accurately reflect quantity of the plasmid (A_{260}/A_{280} ratio is high or 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. Traces of impurities may remain on the binding column after washing and contribute to the absorbance in the final product.
	Plasmid DNA is contaminated with RNA; RNase A treatment is insufficient	Confirm that the RNase A Solution was added to Solution I prior to first use. The RNase A solution may degrade due to high temperatures (>65 °C) or prolonged storage (> 6 months at room temperature).
	Background reading is high due to silica fine particulates	Spin the eluted plasmid DNA sample at maximum speed for 1 minute; use the supernatant to repeat the absorbance readings.
	Column was not dried completely	Ensure wash buffers do not touch column after drying step. Discard wash buffers and repeat centrifugation step to dry column.
	Purification is incomplete due to column overloading	Reduce culture input.

Symbols

The following symbols may appear in the instructions for use or on the packaging and labeling:

Picture	Description
	No additional hazards or not classified as hazardous according to GHS
 MM/DD/YYYY	Use-by date
	Check components for storage conditions.
	Lot number
	Manufacturer
	Website
	Telephone
	Fax
	Email
	LinkedIn
	Twitter
	Facebook

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

AVAILABLE FORMATS


Spin Columns

96-Well Silica Plates


Mag Beads

SAMPLE TYPES


Blood / Plasma

Plasmid


Cultured Cells


Plant & Soil


NGS Clean Up


Tissue


FFPE


Fecal Matter

omega BIO-TEK

innovations in nucleic acid isolation

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