omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

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

D6944-00	5 preps
D6944-01	50 preps
D6944-02	200 preps

Manual Date: February 2022
Manual Revision: v1.0

For Research Use Only

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Introduction

The E.Z.N.A.® FastFilter Plasmid DNA Mini Kit is designed for rapid purification of high-quality plasmid DNA from 1-5 mL bacterial cultures following alkaline-lysis method in just 9 minutes. The Kit features an innovative and first of its kind FastFilter mini column that sits on top of a regular HiBind® silica mini spin column to combine lysate clearance and DNA binding into one simple centrifugation step. 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, 1- 5mL 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 sitting on top of a Hi-Bind® 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 column in one quick step. FastFilter mini column is discarded and the HiBind 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.

Kit Contents

Product	D6944-00	D6944-01	D6944-02
Purifications	5	50	200
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	5	50	200
FastFilter Mini Column	5	50	200
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	3 x 25 mL
Elution Buffer	2 mL	15 mL	30 mL
RNase A	Pre-Added	100 µL	400 µL
User Manual	✓	✓	✓

Storage and Stability

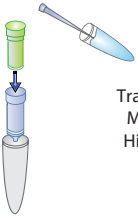
All of the E.Z.N.A.® FastFilter Plasmid DNA Mini Kit components are guarenteed for at least 12 months from date of purchase when stored as follows. Solution I (once RNase A is added) should be stored at 2-8˚C. All other materials should be stored at room temperature. Solution II must be tightly capped when not in use. 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.

Spin Protocol

Pellet by Centrifugation

Resuspend and Lyse

Neutralize



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

Clear Lysate and Bind

Wash 2X

Dry

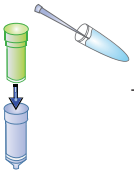
Elute

Vacuum/Spin Protocol

Pellet by Centrifugation

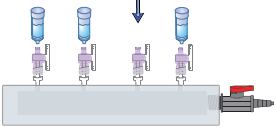
Resuspend and Lyse

Neutralize



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

Clear Lysate and Bind



Wash 2X

Dry

Elute

Preparing Reagents

1. Add the vial of RNase A to the bottle of Solution I and store at 2-8˚C (50 and 200 Purification size 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.

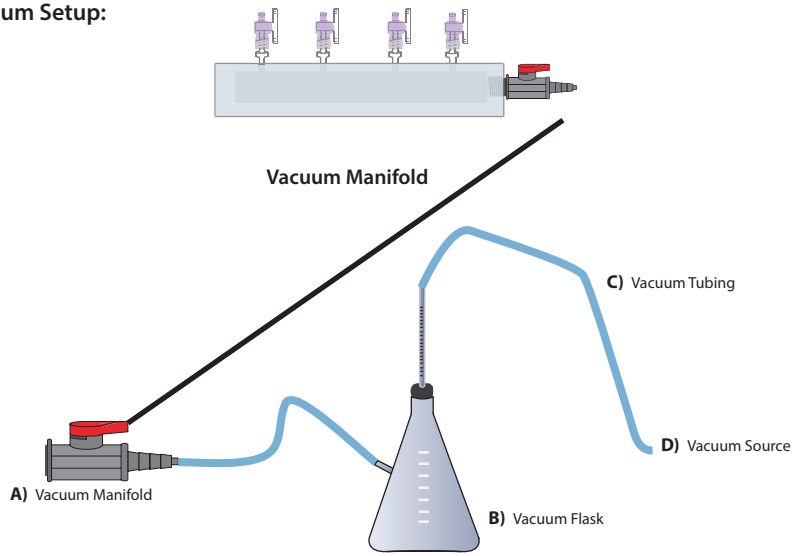
Guidelines for Vacuum Manifold

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:



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 Mini 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, 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.® Plasmid DNA Mini Kits are specially designed for use with cultures grown in Luria Bertani (LB) medium. Richer broths such as TB (Terrific Broth) or 2xYT 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 Mini 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.® FastFilter Plasmid DNA Mini Kit - Spin Protocol

All centrifugation should be performed at room temperature unless otherwise noted. This protocol is designed to isolate plasmid DNA from *E. coli* grown in an overnight 1-5 mL LB culture.

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:

- 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 5.

1. Isolate a single colony from a freshly streaked selective plate, and inoculate a culture of 1- 5 mL LB medium containing the appropriate selective antibiotic. Incubate for ~12-16 hours at 37°C with vigorous shaking (~ 300 rpm). Use a 10-20 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 DH5a® and JM109®.

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

3. Decant or aspirate and discard the culture media.

4. Add 180 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 5.

5. Add 180 µL Solution II. Invert and gently rotate the tube several times to obtain a clear lysate. A 2-3 minute incubation may be necessary.

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

6. Add 90 µL N3 Buffer. Immediately invert several times until a white flocculent precipitate forms.

7. Add 160 µL GBT Buffer. Immediately invert several 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 supernatant from Step 7 by aspirating it into the FastFilter Mini Column.

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

12. Remove the FastFilter Mini Column from the HiBind® DNA Mini Column and discard. Discard the filtrate and reuse the collection tube.

13. Add 200 µL HBC Buffer.

    Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see instructions in the "Preparing Reagents" section on Page 5.

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

15. 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 5.

16. Centrifuge at 13,000*g* for 2 minutes.

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

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

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

19. 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.

20. Store DNA at -20°C.

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

All centrifugation should be performed at room temperature unless otherwise noted.. This protocol is designed to isolate plasmid DNA from *E. coli* grown in an overnight 1-5 mL LB culture. See page 6 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:

- 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 5.

1. Isolate a single colony from a freshly streaked selective plate, and inoculate a culture of 1- 5 mL LB medium containing the appropriate selective antibiotic. Incubate for ~12-16 hours at 37°C with vigorous shaking (~ 300 rpm). Use a 10-20 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 DH5a® and JM109®.

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

3. Decant or aspirate and discard the culture media.

4. Add 180 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 5.

5. Add 180 µL Solution II. Invert and gently rotate the tube several times to obtain a clear lysate. A 2-3 minute incubation may be necessary.

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

6. Add 90 µL N3 Buffer. Immediately invert several times until a flocculent white precipitate forms.

7. Add 160 µL GBT Buffer. Immediately invert several times to mix.

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

9. Insert a FastFilter Mini Column into the HiBind DNA Mini column to the vacuum manifold.

10. Transfer the cleared supernatant from Step 7 by aspirating it into the FastFilter Mini Column.

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

12. Turn off the vacuum. Remove and discard the FastFilter Mini Column.

13. 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 5.

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

15. Turn off the vacuum.

16. 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 5.

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

18. Turn off the vacuum.

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

20. 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.

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

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

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

23. 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.

24. Store DNA at -20°C.

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 DNA eluted	Poor cell lysis	Prepare DNA Wash Buffer according to instructions on Page 5.
	HBC Buffer not diluted with isopropanol	Prepare HBC Buffer according to instructions on Page 5.
Problem	**Cause**	**Solution**
Low DNA Yields	Poor cell lysis	Only use LB or YT medium containing ampicillin. Do not use more than 5 mL (high copy number plasmids) or 10 mL (low copy number plasmids) culture with the basic protocols.
		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 37ºC. 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 column prior to loading the sample. Centrifuge at 10,000*g* for 30 seconds. Discard the filtrate.

Troubleshooting Guide

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**
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 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.
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 cell longer than 16 hours.

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

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