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Introduction

The E.Z.N.A.™ family of products is an innovative system that radically simplifies extraction and purification of nucleic acids from a variety of sources. Key to the system is the Omega Bio-tek's proprietary HiBind™ matrix that avidly, but reversibly, binds DNA or RNA under certain optimal conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or low salt buffer.

E.Z.N.A.® M13 Kits are designed to purify up to 10µg of single-stranded DNA from up to 3ml of phage supernatant. Yields of single-stranded DNA obtained using E.Z.N.A.® M13 Kit is around 3-10 µg and reproducible when the isolations are performed from same culture. M13 infected bacterial cultures are centrifuged to remove bacterial cells, and the culture supernatants are mixed with MPG buffer to precipitate the phage particles. Centrifuge and retain the particle pellet, and after a few steps of purification ,apply the solution to HiBind column. The specially designed membrane will retain intact phage particles. At last, pure ssDNA is eluted with TE or water.

Benefits

The E.Z.N.A.® M13 DNA Isolation Kits mean:

- Speed - M13 DNA isolation in <30 mins
- Reliability - optimized buffers guarantee pure DNA
- Safety - No organic extractions
- Quality - purified DNA suitable for any application

Storage and Stability

All E.Z.N.A.™ M13 DNA Isolation Kit components are guaranteed for at least 12 months from the date of purchase when stored at 22-25°C.

Kit Contents

E.Z.N.A.™ M13 DNA Isolation Kit

Product Number	D6900-00	D6900-01	D6900-02
Purification times	5 Preps	50 Preps	200 Preps
HiBind™ M-13 column	5	50	200
2 ml Collection Tubes	5	50	200
Buffer MPG	5 ml	40 ml	140 ml
Buffer MPB	5 ml	60 ml	240 ml
SPW Wash Buffer Concentrate	2 ml	20 ml	3 x 20 ml
Instruction Booklet	1	1	1

Before Starting

Briefly examine this booklet and become familiar with each step. Prepare all components and have the necessary materials ready before starting.

Supplied By User	Microcentrifuge capable of at least 10,000 x g. Sterile 1.5 or 2 ml centrifuge tubes. Sterile deionized water (or TE buffer) Water bath preheated at 60°C Absolute (96%-100%) ethanol
IMPORTANT	**SPW Wash Buffer Concentrate is to be diluted with absolute ethanol as follows** D6900-00 Add 8 ml 96-100% ethanol D6900-01 Add 80 ml 96-100% ethanol D6900-02 Add 80 ml 100% ethanol to each bottle ***Store diluted SPW Buffer at room temperature***

Note: All steps must be carried out at room temperature.

E.Z.N.A.™ M13 DNA Isolation Kit Spin Protocol

1. **Prepare 4 ml infected M13 culture following stand procedure. The culture should be incubated for 6-7 hours at 37°C with vigorous shaking.**
2. **Pellet bacteria by centrifugation at 5,000 rpm for 15 min at room temperature.**
3. **Transfer the 1-3 ml supernatant containing M13 bacteriophage to a fresh microcentri-fuge tube.** Be careful not to disturb the bacterial pellet during the transferation.
4. **Add 1/5 volume MPG Buffer and mix by gently inverting the tube 10-15 times and incubate at room temperature for 10-15 minutes**.
5. **Place the HiBind® M-13 column in a 2 ml collection tube and apply 700 µl of sample to the column.**
6. Centrifuge at 10, 000 rpm for 30 seconds and discard the flow-through from the collection tube.
7. **Repeat the step 5 and 6 until all sample has been loaded into the column.**
8. **Lyse and bind the DNA onto membrane by adding 500 µl MPB buffer to HiBind® M-13 column.**
9. Centrifuge 30 seconds at 10,000 rpm. Discard the flow-through and reuse the collection tube.
10. **Apply another 500 µl MPB buffer to the column. Incubate for 1 minute at room temperature.** Centrifuge for 30 seconds at 10,000 rpm. Discard the flow-through from collection tube, reuse the collection tube.
11. **Add 700 µl SPW Wash Buffer diluted with absolute ethanol** to the column and centrifuge for 30 seconds at 10,000 rpm.

    Note: SPW Buffer Concentrate must be diluted with absolute ethanol before use. See label for instructions
12. Discard the flow-though liquid and Wash the column with **another 700 µl SPW Buffer by repeating step 11.**

13. Discard the flow-through liquid from collection tube. **Place the column into the collection tube and centrifuge at maxi speed (≥13,000 rpm) for 2 minute to dry the column.**

14. Place the HiBind® M-13 column in a clean 1.5 ml microcentrifuge tube. **Add 30-50 µl TE buffer or water (preheated at 60°C) to the center of the membrane.** Incubate for 1-2 minutes and centrifuge for 1 minute at ≥13,000 rpm. This represents approximately 75-80% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration. The pH of the elution solution can significantly effect the elution efficiency, make sure the pH of the water or TE buffer is between 7.5 -8.0.

15. **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.

E.Z.N.A.™ M13 DNA Isolation Kit Vacuum/Spin Protocol

Note: Please read through previous section of this book before using this protocol.

1. **Prepare the vacuum manifold according to manufacturer' s instructions and connect the V-Spin column to the manifold.**

2. **Load 700 µl of MPG/M-13 supernatant mixture from step 4** on page 4 to the HiBind® M-13 column.

3. Switch on vacuum source to draw the sample through the column and turn off the vacuum.

4. Repeat 2-3 by transferring the remaining mixture into HiBind® M-13 column.

5. **Lyse and bind the DNA onto membrane by adding 500 µl MPB buffer to HiBind® M-13 column.**

6. Switch on vacuum source to draw the sample through the column and turn off the vacuum.

7. **Apply another 500 µl MPB buffer to the column. Incubate for 1 minute at room temperature.** Switch on vacuum source to draw the sample through the column and turn off the vacuum.

8. **Wash the column by adding 700 µl SPW wash buffer diluted with absolute ethanol**. Draw the wash buffer through the column by turning on the vacuum source. Repeat this step with another 700 µl SPW wash buffer.

   Note: SPW Buffer Concentrate must be diluted with absolute ethanol before use. See label for instructions

9. **Assemble the column into a 2 ml collection tube and transfer the column to a microcentrifuge. Spin at maximum speed for 2 minute to dry the column.**

10. **Place the HiBind® M-13 column in a clean 1.5 ml microcentrifuge tube. Add 30-50 µl TE buffer or water (preheated at 60°C)** to the center of the membrane. Incubate for 1-2 minutes and centrifuge for 1 minute at 10,000 rpm.

Trouble Shooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Incorrect host stain	Make sure that host strain carries the F'-episome, which is essntial for M13 infection.
	Bacterial culture overgrown or not fresh.	Do not incubate cultures for more than 8 hr at 37°C.
	Lower pH on the elution buffer	Make sure the pH of the elution solution is between 7.5-8.0
	Elution buffer did not cover the membrane complately	Make sure that elution buffer is dispensed directly onto the center of the membrane
	Column clogged	use less than 3 ml M13 phage supernatant per column.
No DNA eluted.	SPW Buffer Concentrate not diluted with absolute ethanol.	Prepare SPW Buffer Concentrate as instructed above.
High molecular weight DNA contamination of product.	Carryover the bacterial cell during transfer	Make sure not carry bacterial during the transfer of the supernatant. A extra centrifugation step may be necessary.
Optical densities do not agree with DNA yield on agarose gel.	Trace contaminants eluted from column increase A_{260}.	Make sure to wash column as instructed , rely on agarose gel/ethidium bromide electrophoresis for quantitation.
M13 DNA floats out of well while loading agarose gel	Ethanol not completely removed from column following wash steps.	Centrifuge column as instructed in step 13 to dry .

Ordering Information

Product	Applications	Cat. No.
Standard E.Z.N.A.™ Plasmid Isolation System		
Plasmid Mini Kit I	Isolation of up to 30 µg Plasmid DNA	D6942/3
Plasmid Mini Kit II	Isolation of up to 70 µg Plasmid DNA	D6945
Plasmid Midi Kit	Isolation of up to 250 µg Plasmid DNA	D6904
Plasmid Maxip Kit	Isolation of up to 1.5 mg Plasmid DNA	D6922
Fastfilter Plasmid Midi Kit	Isolation of up to 250 µg Plasmid DNA, featuring filter syringes for lysate clearance	D6905
Fastfilter Plasmid Maxi Kit	Isolation of up to 1.5 mg Plasmid DNA, featuring filter syringes for lysate clearance	D6924
E-Z 96 Fastfilter Plasmid Kit	Isolation of Plasmid DNA using a 96-well format	D1097
E-Z 96 SE Plasmid Kit	Isolation of plasmid DNA using a single plate	D1095
Yeast Plasmid Isolation Kit	Isolation fo Yeast Plasmid DNA	D3476
E.Z.N.A.™ Endotoxin Free Plasmid Isolation System		
Endo-Free Plasmid Mini Kit I	Isolation of up to 30 µg Endotoxin free Plasmid	D6948
Endo-Free Plasmid Mini Kit II	Isolation of up to 70 µg Endotoxin free Plasmid	D6950
Endo-Free Plasmid Mid Kit	Isolation of up to 250 µg Endotoxin free Plasmid DNA, featuring filter syringes for lysate clearance	D6915
EndoFree Plasmid Maxi Kit	Isolation of up to 1.5 mg Endotoxin free Plasmid DNA, featuring filter syringes for lysate clearance	D6926
E.Z.N.A.™ H P Plasmid Isolation System		
HP Plasmid Mini Kit I	Isolation of up to 30 µg of High Purity Plasmid	D7042
HP Plasmid MidiKit	Isolation of up to 200 µg of High Purity Plasmid	D7004
HP Plasmid Maxi Kit	Isolation of up to 1.5 mg of High Purity Plasmid	D7022
E.Z.N.A.™ Single Strand Phage DNA Isolation Kits		
M13 Isolation Kit	Isolation of up to 15µg of single stranded phage	D6900
E-Z 96 M13 Isolation Kit	Isolation of up to 15µg of M-13 DNA using a 96-	D1900
E.Z.N.A.™ Large Construct DNA Isolation Kits		
BAC/PAC DNA Isolation Kit	Effective purification of BAC or PAC DNA	D2156
BAC/PAC DNA Isolation Kit	purification of BAC or PAC using a 96-well format	D1056