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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 Kits are 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. The samples are then loaded to HiBind™ M13 columns or M13 96-well plates. The specially designed membrane will retain intact phage particles. Phage particles are lysed and bind to the membrane after adding the MPB buffer. Contaminants such as protein are efficiently wash away with DNA Wash buffer, and pure ssDNA is eluted with TE or water.

## Benefits

The E.Z.N.A.® M13 Kits mean:

- Speed - M13 DNA isolation in <30 min
- 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 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 Kit

Product Number	D6900-00	D6900-01	D6900-02
Purification	5	50	200
HiBind™ M13 Mini-column	5	50	200
2 ml Collection Tubes	5	50	200
MPG Buffer	1 ml	20 ml	80 ml
MPB Buffer	5 ml	80 ml	400 ml
Wash Buffer Concentrate	12 ml	40 ml	3 x 40 ml
Instruction Booklet	1	1	1

### E-Z 96™ M13 Kit

Product Number	D1900-01	D1900-02
<b>Purification</b>	<b>4 x 96</b>	<b>20 x 96</b>
HiBind™ M13 96-well plate	4	20
300 µl collection plate	4	20
1.2 ml 96-well Tube rack	4	20
800 µl Collection Plate	1	1
2 ml Collection Plate	1	1
MPG Buffer	120 ml	600 ml
MPB Buffer	600 ml	3000 ml
Wash Buffer Concentrate	3 x 40 ml	6 x 100 ml
Instruction Booklet	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** Microcentrifuge capable of at least 10,000 x g.

**User:** Sterile 15 ml centrifuge tubes.

Sterile 1.7 ml centrifuge tubes..

Sterile deionized water (or TE buffer)

Water bath preheated at 60° C

Absolute (96%-100%) ethanol

Centrifuge with swinging-bucket rotor at room temperature capable of 4000 x g (such as Eppendorf 5810 with MTP rotor) for M13 96-well kit).  
Vacuum Manifold (Omega Product # Vac-3) (for M13 96-well kit)

### IMPORTANT

**DNA Wash Buffer Concentrate is to be diluted with absolute ethanol as follows:**

D6900-01 and D6900-02	Add 60 ml 100% ethanol to each bottle
D1900-01 and D1900-02	Diluted with 150 ml 100% ethanol

***Store diluted DNA Wash Buffer at room temperature***

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

## E.Z.N.A.™ M13 Miniprep 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 4,000 rpm for 15min at room temperature.
3. Transfer 1.4 ml supernatant containing M13 bacteriophage to a fresh 1.7 ml microcentrifuge tube. **Be careful not to disturb the bacterial pellet during the transfer.**
4. Add 200µl MPG Buffer with the M13 supernatant and mix by vortexing and incubate at room temperature for 10-15 minutes.
5. Place the HiBind® M13 Minicolumn 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 step 5 and 6 until the entire sample has been loaded onto the column.
8. Lyse and bind the DNA onto membrane by adding 700 µl MPB buffer to the HiBind® M13 Minicolumn. Incubate for 1 minute at room temperature.
9. Centrifuge for 30 seconds at 10,000 rpm. Discard the flow-through and reuse the collection tube.
10. Apply another 700 µl MPB buffer to the column and centrifuge for 15 seconds at 10,000 rpm. Discard the flow-through from collection tube and reuse the collection tube.
11. Add 700 µl DNA wash buffer and centrifuge for 30 seconds at 10,000 rpm. **Note:** Wash Buffer Concentrate must be diluted with absolute ethanol before use. See label for instructions
12. Discard the flow-through and Wash the column with another 700 µl DNA Wash buffer by repeating step 11.
13. Discard the flow-through from collection tube. Place the column into the collection tube and centrifuge at 10,000 rpm for 1 minute to dry the column.
14. Place the HiBind® M13 Minicolumn in a clean 1.7 ml microcentrifuge tube.

Add 80-100 µl TE or water (preheated at 60° C ) to the center of the membrane. Incubate for 10 minutes and centrifuge for 1 minute at 10,000 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 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 96™ M13 Protocol

1. Grow M13 infected bacterial in 2.2 ml 96-well culture plate.
2. Spin down bacterial cells by centrifugation at 4000 rpm for 15 minutes at room temperature.
3. Transfer supernatant to fresh tube rack. **Be careful not to disturb the bacterial pellet during the transfer.**
4. Add 200µl MPG Buffer with the M13 supernatant and mix by vortexing and incubate at room temperature for 15 minutes.
5. Assemble the vacuum manifold by following instruction from manufacturer.
6. Add 1 ml of the samples to each well of E-Z 96™DNA Plate. Switch on vacuum for 2 minutes to draw through the sample.
7. Add 800 µl of buffer MPB to each well of the E-Z 96™DNA Plate. Incubate 2 minutes and apply the vacuum until all samples pass through membrane.
8. Stop the vacuum and add another 800 µl of buffer MPB to each well of the E-Z 96™DNA Plate. Incubate 2 minutes at room temperature.
9. Apply the vacuum until all the liquid passes through the membrane.
10. Apply 1.2 ml DNA wash buffer to each well of the E-Z 96™DNA Plate and switch on the vacuum until all liquid has been drawn through the membrane.

11. Remove the E-Z 96™ DNA Plate from the vacuum manifold and remove any trace of liquid by tapping the E-Z 96™ DNA Plate firmly on a stack of paper towels. Visually inspect the walls of wells and make sure that all droplets are removed by tapping.
12. Place the E-Z 96™ DNA Plate back on the manifold and apply the vacuum for another 5 minutes. Repeat the tapping step from step 11 to completely remove any remain liquid.
13. Optional: Place the E-Z 96™ DNA Plate into a vacuum oven preset on 60°C and incubate 10 minutes to completely dry the plate.
14. Assemble the vacuum manifold by placing 800ul 96-well collection plate in the base of the Vacuum manifold and place the 300ul collection plate on top the 800ul plate. Close the top part of the manifold. Place the E-Z 96™ DNA Plate on top of the manifold.
15. Add 80-100 µl TE or water to the center of the membrane in each well of the E-Z 96™ DNA Plate. Incubate 10 minutes. Apply the vacuum for 5 minutes to elute DNA.

### Trouble Shooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Incorrect host stain	Make sure that host strain carries the F'-episome, which is essential 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 completely	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.	Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare Wash 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 9 to dry .