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Introduction

The E.Z.N.A.™ family of products is an innovative system that radically simplifies the extraction and purification of nucleic acids from a variety of sources. The key to this system is Omega Bio-Tek, Inc.'s (OBI) new HiBind® matrix that specifically, but reversibly, binds DNA or RNA under certain optimal conditions, while allowing proteins and other contaminants to be removed. The nucleic acids bound to OBI's HiBind® Matrix are easily eluted with deionized water or a low salt buffer, and then suitable for any downstream application.

OBI's 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 the same culture.

The E.Z.N.A.™ M13 procedure first calls for the infected bacterial culture to be centrifuged to pellet the bacterial cell, and then MPG buffer is added to the supernatent to precipitate the phage particles. Next, the samples are loaded on to HiBind® columns or on to E-Z® 96 plates. The specially designed HiBind® matrix will retain intact phage particles. These phage particles will then be lysed and bound to the HiBind® membrane after the addition of MPX Buffer. Finally, contaminants such as protein are efficiently washed away with DNA Wash buffer, and pure ssDNA is eluted with TE or water.

Benefits

Omega Bio-Tek, Inc.'s E.Z.N.A.™ M13 & E-Z 96® M13 Kits Signify:

- Speed -The Isolation of M13 DNA in < 30 min
- Reliability -Optimized Buffers Guarantee Pure DNA Each Time
- Safety- No Organic Extractions
- Quality-Purified DNA is Suitable for any Downstream Application

Kit Contents

E.Z.N.A.™ M13 Isolation Kit

Product Number	D6900-00	D6900-01	D6900-02
Purification times	5 Preps	50 Preps	200 Preps
HiBind® M13 Minicolumn	5	50	200
2 mL Collection Tubes	5	50	200
MPG Buffer	1 mL	20 mL	80 mL
MPX Buffer	5 mL	80 mL	300 mL
SPW Wash Buffer Concentrate	5 mL	20 mL	3 x 25 mL
Elution Buffer	3 mL	10 mL	30 mL
Instruction Booklet	1	1	1

E-Z 96® M13 Isolation Kit

Product Number	D1900-00	D1900-01	D1900-02
Purification times	1 x 96	4 x 96	20 x 96
E-Z 96 ® DNA Plate	1	4	20
Caps for Racked Collection Tubes	12 x 8	48 x 8	240 x 8
Racked Collection Tubes (1.2ml)	1 x 96	4 x 96	20 x 96
2 ml Collection Plate	1	4	20
MPG Buffer	40 mL	180 mL	900 mL
MPX Buffer	200 mL	900 mL	4500 mL
SPW Wash Buffer Concentrate	25 mL	100 mL	3 x 200 mL
Elution Buffer	20 mL	80 mL	220 mL
Instruction Booklet	1	1	1

Storage and Stability

All E.Z.N.A.™ or E-Z 96® M13 Kit components are guaranteed for at least 12 months from the date of purchase when stored at 22-25°C.

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:

E.Z.N.A.™ M13 Isolation Kit

- Microcentrifuge capable of at least 10,000 x g
- 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

E-Z® 96 M13 Isolation Kit

- Centrifuge with swinging-bucket rotor at room temperature capable of 5000 x g.
- Adaptor for microplate
- Vacuum Manifold (Omega Product # Vac-03) (for 96-well plate format kit)

IMPORTANT	**SPW Wash Buffer Concentrate is to be diluted with absolute ethanol (~96-100%) as follows:**
	D6900-00 Add 20 mL absolute ethanol
	D6900-01 Add 80 mL absolute ethanol
	D6900-02 Add 100 mL absolute ethanol to each bottle
	D1900-00 Add 100 mL absolute ethanol
	D1900-01 Add 400 mL absolute ethano
	D1900-02 Add 800 mL absolute ethanol to each bottle
	Store diluted SPW Wash Buffer at room temperature

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

E.Z.N.A.™ M13 Isolation Protocol

Product Number D6900

1. **Prepare 4 mL of an infected M13 culture, and incubate with vigorous shaking for 6-7 hours at 37 °C .**

2. Pellet bacteria by centrifugation at 5,000 rpm for 15 min at room temperature.

3. **Transfer 1.4 mL of the supernatant obtained containing the M13 bacteriophage, into a fresh reaction tube.** Be careful not to disturb the bacterial pellet during the transfer. If the supernatant is not clear, repeat the centrifugation step.

4. **Add 280 ul of MPG Buffer to the M13 supernatant and mix by vortexing.** Then, incubate at room temperature for 10-15 minutes.

5. **Place OBI's HiBind® M13 Minicolumn into a 2 mL collection tube and add 700 µL of the M13 sample to the column.**

6. Centrifuge at 10, 000 rpm for 30 seconds and discard the flow-through collected in the collection tube.

7. **Repeat steps 5 and 6 until all of the sample has been passed through the HiBind® M13 Minicolumn.**

8. **Lyse and bind the DNA onto the membrane by adding 700 µL of MPX buffer to the HiBind® M13 Minicolumn.**

9. Immediately centrifuge for 30 seconds at 10,000 rpm. Discard the flow-through and reuse the collection tube.

10. **Apply another 700 µL of MPX buffer to the HiBind® M13 Mini-column and incubate at room temperature for 1 minute.** Centrifuge for 30 seconds at 10,000 rpm. Discard the flow-through from the collection tube and reuse the collection tube.

11. Add 700 µL of SPW Wash Buffer diluted with absolute ethanol to the HiBind® M13 Minicolumn, and centrifuge it for 30 seconds at 10,000 rpm.

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

12. **Discard the flow-though and wash the column with another 700 µL of SPW Wash Buffer by repeating step 11.**

13. Discard the flow-through in the collection tube, and reuse the collection tube by placing the column into the collection tube and centrifuging at maximal speed (≥13,000 rpm) for 1 minute in order to dry the column.

14. Place the dried HiBind® M13 Minicolumn into a clean 1.5 mL microcentrifuge tube, and add 50-100 µL of Elution Buffer (preheated at 60°C, 10mM Tris, pH8.5) to the center of the membrane. Incubate at room temperature for 10 minutes and centrifuge for 1 minute at maximal speed (≥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 also significantly affect 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 Isolation Vacuum Manifold Protocol

Product Number D1900

1. Grow the M13 infected bacteria in a 2.2 mL 96-well culture plate (not supplied).

2. Spin down bacterial cells by centrifugation at 5000 rpm for 15 minutes at room temperature.

3. Transfer 1-2 mL of the supernatant into a 2 mL Collection Plate (supplied). ***Be careful not to disturb the bacterial pellet during the transfer. If the supernatant is not clear, repeat the centrifugation step.***

4. **Add 1/5 volume of MPG Buffer (200µl MPG per 1 mL culture) to the M13 supernatant and mix by vortexing**. Incubate at room temperature for 15 minutes.

5. Assemble the vacuum manifold by following the manufacturer's instructions. If using Vac-03 vacuum manifold, place the waste collection tray inside the manifold and place the E-Z® 96 DNA Plate on top part of the manifold.

6. Transfer 1 mL of the cleared supernatant from step 4 into each well of the E-Z® 96 DNA Plate. Switch on the vacuum for 2 minutes to draw the sample through. Note: Load 1mL of the sample at a time. If the volume of the sample is more than 1mL, ventilate the vacuum manifold and load another 1mL of the sample into each well of the E-Z® 96 DNA Plate.

7. **Add 1 mL of buffer MPX to each well of the E-Z 96® DNA Plate**. Immediately apply the vacuum to draw all of the samples through the membrane.

8. **Stop the vacuum and add another 1 mL of buffer MPX to each well of the E-Z 96® DNA Plate.** Incubate for 2 minutes at room temperature.

9. Next, apply the vacuum until all of the liquid passes through the membrane.

10. Add 1 mL of SPW Wash Buffer into each well of the E-Z 96® DNA Plate and switch on the vacuum until all of the liquid has passed through the membran.

11. Remove the E-Z 96® DNA Plate from the vacuum manifold and remove any traces of liquid by tapping the E-Z® 96 DNA Plate firmLy on a stack of paper towels. Visually inspect the walls of the 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 remaining liquid.

13. Optional: Place the E-Z® 96 DNA Plate into a vacuum oven preset at 60°C and incubate for 10 minutes to completely dry the plate.

14. Re-assemble the vacuum manifold by now replacing the waste collection tray with a 1.2 mL rack of microtubes (supplied). Place the E-Z® 96 DNA Plate on the top part of the manifold.

15. Add 75-150 µL of preheated (60°C) Elution Buffer (10mM Tris-HCl, pH 8.5) into the center of the membrane to each well of the E-Z® 96 DNA Plate. Incubate for 10 minutes. Apply the vacuum for 5 minutes to elute DNA. 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 also significantly affect the elution efficiency , therefore make sure that the pH of the water or TE is between 7.5 -8.0.

E-Z 96® M13 Isolation Spin Protocol

Product Number D1900

1. Grow the M13 infected bacteria in a 2.2 mL 96-well culture plate (not supplied). Spin down bacterial cells by centrifugation at 5000 rpm for 15 minutes at room temperature.

2. Transfer 1mL of the supernatant into a 2 mL Collection Plate (supplied). Be careful not to disturb the bacterial pellet during the transfer. If the supernatant is not clear, repeat the centrifugation step.

3. **Add 1/5 volume of MPG Buffer (200µl MPG per 1 mL culture) to the M13 supernatant and mix by vortexing**. Incubate at room temperature for 10-15 minutes.

4. Transfer 1 mL of the cleared supernatant from step 3 into each well of the E-Z® 96 DNA Plate. Place E-Z® 96 DNA Plate onto 2 ml collection Plate with tape or film. Place plate/2mL collection tube together in centrifuge's swing-bucket rotor with adapter for deep well plate. Centrifuge at 3,000 x g for 5 minutes. Discard the liquid.

5. **Add 1 mL of Buffer MPX to each well of the E-Z 96® DNA Plate**. Immediately Centrifuge at 3000 x g for 5 minutes. Discard the flow-through and reuse the 2 mL collection tubes for next step.

6. **Add another 1 mL of buffer MPX to each well of the E-Z 96® DNA Plate.** Incubate for 2 minutes at room temperature. Centrifuge at 3000 x g for 5 minutes. Discard the flow-through and reuse the 2 mL collection tube for next step.

7. Add 1 mL of SPW Wash Buffer into each well of the E-Z 96® DNA Plate. Centrifuge at 3000 x g for 15 minutes. Discard the flow-through and reuse the 2 mL collection tube for next step.

8. **(Optional)** Place E-Z 96® DNA Plate into a vacuum oven or incubator which was preset to 70°C for 10 minutes. This step will ensure that the DNA plate is completely dried before DNA elution.

9. Carefully place the E-Z 96® DNA plate on top of the new Racked Microtubes (supplied). **Add 75-150 µl Water or Elution Buffer (10mM Tris, pH8.5) to each well of the E-Z 96® DNA Plate**. Let stand for 2 minutes.

10. Centrifuge at 3000 x g for 5 minutes to elute DNA and seal the tube with caps. This represents approximately 75-80% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

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. Avoid the bacterial pellet during transfer.
No DNA eluted.	SPW Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare SPW Wash Buffer Concentrate as instructed above.
High molecular weight DNA contamination of product.	Carryover of the bacterial cell during transfer	Make sure not carry any bacteria during the transfer of the supernatant. An 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, and 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 .