omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® M13 DNA Mini Kit

D6900-00	5 preps
D6900-01	50 preps

Manual Date: May 2019
Revision Number: v4.0

For Research Use Only

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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's HiBind® matrix that specifically, but reversibly, binds DNA or RNA under optimal conditions, while allowing proteins and other contaminants to be removed. The nucleic acids bound to the HiBind® matrix are easily eluted with deionized water or a low salt buffer. DNA and RNA are suitable for many downstream applications.

Omega Bio-tek's E.Z.N.A.® M13 DNA Kits are designed to purify up to 10 µg of single-stranded DNA from up to 3 mL of phage supernatant. Yields of single-stranded DNA obtained using E.Z.N.A.® M13 DNA Kits are around 3-10 µg and reproducible when the isolations are performed from the same culture.

Overview

The E.Z.N.A.® M13 DNA Kit isolation procedures first call for the infected bacterial culture to be centrifuged to pellet the bacterial cells. MPG buffer is added to the supernatant to precipitate the phage particles. The samples are loaded on HiBind® M13 DNA Mini Columns. The specially designed HiBind® matrix will retain intact phage particles. These phage particles are lysed and bound to the HiBind® matrix after the addition of MPX Buffer. Contaminants such as protein are efficiently washed away with SPW Buffer and pure ssDNA is eluted with Elution Buffer.

New in this Edition:

May 2019
- D1900, all prep sizes, have been discontinued and are no longer available for purchase.
- SPW Wash Buffer has been renamed SPW Buffer. This is a name change only. The formulation has not changed.

June 2013
- This manual has been edited for content and redesigned to enhance user readability.

Kit Contents

Product	D6900-00	D6900-01
Purifications	5 preps	50 preps
HiBind® M13 DNA Mini Column	5	50
2 mL Collection Tubes	5	50
MPG Buffer	2 mL	20 mL
MPX Buffer	8 mL	80 mL
SPW Buffer	5 mL	25 mL
Elution Buffer	15 mL	30 mL
User Manual	✓	✓

Storage and Stability

All E.Z.N.A.® M13 DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored at room temperature.

Preparing Reagents

Dilute SPW Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D6900-00	20 mL
D6900-01	100 mL

E.Z.N.A.® M13 Miniprep Kit Protocol

Materials and Equipment to be Supplied by User:

- 100% ethanol
- Microcentrifuge capable of at least 12,000 x *g*
- Water bath or incubator capable of 65°C
- Nuclease-free 1.5 mL or 2.0 mL microcentrifuge tubes

Before Starting:

- Prepare SPW Buffer according to the instructions on Page 3.
- Heat the Elution Buffer to 65°C.

1. Prepare a 4 mL culture of infected M13.

2. Incubate at 37°C for 6-7 hours with vigorous shaking.

3. Centrifuge at 5,000 rpm for 15 minutes at room temperature.

4. Transfer 1.4 mL of the supernatant obtained containing the M13 bacteriophage, into a fresh reaction tube.

   Note: Be careful not to disturb the bacterial pellet during the transfer. If the supernatant is not clear, repeat the centrifugation step.

5. Add 280 µL MPG Buffer to the M13 supernatant and mix by vortexing.

6. Let sit at room temperature for 10-15 minutes.

7. Add 700 µL sample to a HiBind® M13 DNA Mini Column inserted into a 2 mL Collection Tube.

8. Centrifuge at 10,000 rpm for 30 seconds. Discard the filtrate and reuse the collection tube.

9. Repeat Steps 7 and 8 until all of the sample has been passed through the HiBind® M13 DNA Mini Column.

10. Add 700 µL MPX Buffer.

11. Centrifuge for 30 seconds at 10,000 rpm.

12. Discard the filtrate and reuse the collection tube.

13. Add 700µL MPX Buffer.

14. Let sit for 1 minute at room temperature.

15. Centrifuge at 10,000 rpm for 30 seconds.

16. Discard the filtrate and reuse the collection tube.

17. Add 700 µL SPW Buffer.

    Note: SPW Buffer must be diluted with 100% ethanol before use. Please see Page 3 for instructions.

18. Centrifuge at 10,000 rpm for 30 seconds.

19. Discard the filtrate and reuse the collection tube.

20. Repeat Steps 17-19 for a second SPW Buffer wash step.

21. Centrifuge the empty column at maximum speed for 1 minute.

22. Insert the HiBind® M13 DNA Mini Column into a clean 1.5 mL microcentrifuge tube (not provided).

23. Add 50-100 µL Elution Buffer heated to 65°C.

    Note: Make sure to add Elution Buffer directly onto the HiBind® M13 DNA Mini Column matrix.

24. Let sit at room temperature for 10 minutes.

25. Centrifuge at maximum speed for 1 minute.

26. Store eluted 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
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.
Problem	**Cause**	**Solution**
No DNA eluted	SPW Buffer not diluted with ethanol.	Prepare SPW Buffer as instructed on Page 4.
High-molecular weight DNA contamination of product	Carryover of the bacterial cell during transfer.	Make sure not to 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 A260.	Make sure to wash column as instructed, and rely on agarose gel/ ethidium bromide electrophoresis for quantitation.
DNA floats out of well while loading agarose gel	Ethanol not completely removed from column following wash steps.	Centrifuge column as instructed in Step 19 to dry.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
DNase/RNase-free Microcentrifuge Tubes, 1.5 mL, 500/pk, 10 pk/cs	SSI-1210-00
DNase/RNase-free Microcentrifuge Tubes, 2.0 mL, 500/pk, 10 pk/cs	SSI-1310-00
SPW Buffer, 25 mL	PDR045
Elution Buffer, 100 mL	PDR048

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

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