

Mag-Bind™ SE DTR

Table of Contents

Introduction and Principle.....	2
Illustrated Protocol	3
Kit Contents and Storage	4
Mag-Bind™ SE DTR 96 Plate Protocol.....	5
Mag-Bind™ SE DTR 384 Plate Protocol.....	7
Troubleshooting Guide.....	9
Ordering Information	10

Manual Revision: March 2010



Introduction and Principle

Introduction

Excess unincorporated, nonradioactive label can cause high background fluorescence in automated sequencing gels. For optimal sequencing results, remaining labeled dideoxynucleotides should be removed prior to electrophoresis.

Omega Bio-tek's Mag-Bind™ SE DTR (Mag-Bind™ SE **D**ye-**T**erminator **R**emoval) is designed to effectively and reliably remove unincorporated terminators from sequencing reactions. The system combines Omega Bio-Tek's proprietary chemistries with the reversible nucleic acid-binding properties of paramagnetic beads to eliminate excess nucleotides, primers, and small, nontargeted amplification products such as primer dimers. This kit is designed for both manual and fully automated purification of sequencing products.

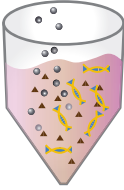
Principle

The Mag-Bind™ SE DTR paramagnetic particles technology provides a better solution for nucleic acid purification than centrifugation and vacuum-based technologies. The product can be easily scaled up while providing simple user-friendly handling procedures. If using the Mag-Bind™ SE DTR for the first time, please read this booklet to become familiar with the procedures. Sequencing products are first mixed with the Mag-Bind™ SE DTR. DNA then selectively binds to the Mag-Bind™ SE DTR particles. With one rapid wash step, trace contaminants such as nucleotides, primers and small, non-targeted amplification products are removed. Pure DNA is eluted in low salt buffer or water. Purified DNA can be directly used in downstream applications without the need for further purification.

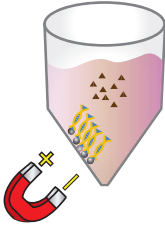
New in this edition:

- The new edition of this manual has been enhanced to improve readability and protocol quality.

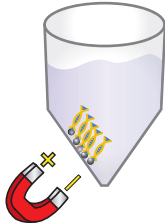
Illustrated Protocol



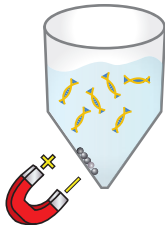
Add Mag-Bind™ SE DTR and 85% Ethanol, Mix



Magnetize and remove cleared supernatant



Wash 2 x with 85% Ethanol



Elute and transfer DNA to a new plate

Kit Contents and Storage

Kit Contents

Product No.	M1300-05	M1300-08	M1300-50
Mag-Bind™ SE DTR	5 ml	50ml	500 ml
Preparations	500* / 1,000**	5,000* / 10,000**	50,000* / 100,000**
User Instruction Manual	1	1	1

* Based on typical 10 μ l reaction volume in a 96 well format.

** Based on typical 5 μ l reaction volume in a 384 well format.

Storage and Stability

Mag-Bind™ SE DTR are stable for at least 9 months from the date of purchase when stored at 2°C- 8°C. **Contents of the kit should not be frozen at any time.**

Mag-Bind™ SE DTR 96 Plate Protocol

Mag-Bind™ SE DTR 96 Plate Sequencing Dye Removal Protocol

Additional Materials Supplied by User:

- 85% ethanol (Prepared from non denatured ethanol)
- Magnetic separation device compatible with 96 PCR Plates
- Multichannel pipet
- Polypropylene reservoirs
- 96 well plate capable of being used in sequencers
- Elution Buffer (0.1mM EDTA or $\text{Di H}_2\text{O}$)

1. Shake the Mag-Bind™ SE DTR bottle to fully resuspend the magnetic beads.
2. Add 10 μl of the Mag-Bind™ SE DTR to each sample.

Note: Use 10 μl of Mag-Bind™ SE DTR regardless of the volume of the sequencing reaction.

3. Add 85% ethanol volume according to table below and mix the sample thoroughly by pipetting up and down 7-10 times.

Note: Do not use denatured ethanol. Always prepare fresh 85% ethanol within 3 days of use and store tightly capped.

Reaction volume (μl)	85% Ethanol (μl)
5	30
10	40
15	50
20	60

4. Place the sample plate on a magnetic separation device for 5-7 minutes or until the solution clears.
5. Aspirate and discard the cleared supernatant.
6. With the plate on the magnet, add 100 μl of 85% ethanol to each well and wait 2-3 minutes or until the magnetic beads is fully resettled. It is not necessary to mix or resuspend the magnetic beads.

Mag-Bind™ SE DTR 96 Plate Protocol

7. Aspirate and discard the supernatant.
8. Add 100µl of 85% ethanol and incubate at room temperature for 1-2 minutes .
9. Aspirate and discard the supernatant. Air dry the magnetic particles for 10 minutes.
Note: It is critical to completely remove all liquid from each well since it contains excess fluorescent dye and other contaminants
10. Add 40 µl appropriate type of Elution Buffer (0.1mM EDTA or Di H₂O). Mix thoroughly by pipetting up and down for 20 times. Incubate at room temperature for 5 minutes.
11. Place the plate onto a magnetic separation device and wait 7-10 minutes or until the magnetic beads are cleared from solution
12. Transfer 30-35 µL of cleared supernatant which contains purified sequencing product into a new plate capable of being used in sequencer.

Mag-Bind™ SE DTR 384 Plate Protocol

Mag-Bind™ SE DTR 384 Plate Sequencing Dye Removal Protocol

Additional Materials Supplied by User:

- 85% ethanol (Prepared from non denatured ethanol)
- 384 well magnetic stand
- Polypropylene reservoirs
- 384 processing plate
- Elution buffer (0.1 mM EDTA or $\text{Di H}_2\text{O}$)

1. Shake the Mag-Bind™ SE DTR bottle to fully resuspend the magnetic beads.

2. Add 5 μl of the Mag-Bind™ SE DTR to each sample.

Note: Use 5 μl of Mag-Bind™ SE DTR regardless of the volume of the sequencing reaction.

3. Add 85% ethanol volume according to table below and mix the sample thoroughly by pipetting up and down 7-10 times.

Note: Do not use denatured ethanol. Always prepare fresh 85% ethanol within 3 days of use and store tightly capped.

Reaction volume (μl)	85% Ethanol (μl)
5	14.3
10	21.4
15	28.6

4. Place the sample plate on a magnetic separation device for 5-7 minutes or until the solution clears.

5. Aspirate and discard the cleared supernatant.

6. With the plate on the magnet, add 30 μl of 85% ethanol to each well and wait 5-7 minutes or until the magnetic beads is fully resettled. It is not necessary to mix or resuspend the magnetic beads.

7. Aspirate and discard the supernatant.

Mag-Bind™ SE DTR 384 Plate Protocol

8. Add 30µl 85% ethanol and incubate at room temperature for 1-2 minutes .
9. Aspirate and discard the supernatant. Air dry the magnetic particles for 10 minutes.
Note: It is critical to completely remove all liquid from each well since it contains excess fluorescent dye and other contaminants
10. Add 15-20 µl appropriate type of Elution Buffer (0.1mM EDTA or Di H₂O). Mix thoroughly by pipetting up and down for 20 times. Incubate at room temperature for 5 minutes.
11. Place the plate onto a magnetic separation device and wait 7-10 minutes or until the magnetic beads are cleared from solution
12. Transfer cleared supernatant which contains purified sequencing product into a new plate capable of being used in sequencer.

Troubleshooting Guide

Please use this guide to solve any problems that may arise. We hope that it will aid in clearing up any questions for you. If for any reason you need further assistance, please contact our technical support staff at our **Toll Free Number (1-800-832-8896)**.

Possible Problems and Suggestions

Problem	Cause	Solution
Dye terminator remain in the eluted DNA and cause blobs.	Supernatant is not removed completely	Making sure to remove any liquid drops from each well of the plate.
	Too much BigDye	Use less BigDye per reaction
	Insufficient Washing	During steps 6-7 mix beads to wash more effectively
Problem	Cause	Solution
Low Signalct Problem	Ethanol concentration is not correct	Make sure to use correct volume of ethanol
	Low ethanol concentration	Check the ethanol concentration, use fresh ethanol if necessary
	Cause	Solution
	Magnetic beads are lost during the process	Make sure not to remove any magnetic beads during aspiration

Ordering Information

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

Buffer (Size)	Part Number
Nuclease Free Water (1mL)	PD092
500 μ L processing plate	EZ9604-02
Mag-Bind™ SE DTR (50mL)	M1300-08
Mag-Bind™ SE DTR (500mL)	M1300-50
Mag-Bind™ E-Z Pure (50mL)	M1380-01
Mag-Bind™ E-Z Pure (500mL)	M1380-02

HiBind, E.Z.N.A and MicroElute are registered trademarks of Omega Bio-tek, Inc.
*PCR is a patented process of Hoffman-La Roche. Use of PCR process requires a license.

Note:

Note: