OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

Mag-Bind® SeqDTR™

M1300-05	5 mL
M1300-08	50 mL
M1300-50	500 mL

January 2013

For research use only.Not intended for diagnostic testing.

Mag-Bind® SeqDTR™

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Manual Revision: January 2013

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Introduction and Overview

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

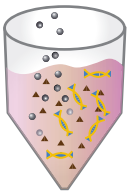
Omega Bio-tek's Mag-Bind® SeqDTR™ is designed to effectively and reliably remove unincorporated terminators from sequencing reactions. The system combines Omega Bio-tek's proprietary chemistries with the reversible nucleic acid-binding properties of magnetic beads to eliminate excess nucleotides, primers, and small, non-targeted amplification products such as primer dimers. This kit is designed for both manual and fully automated purification of sequencing products.

The Mag-Bind® SeqDTR™ magnetic 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® SeqDTR™ for the first time, please read this booklet to become familiar with the procedures. Sequencing products are mixed with the Mag-Bind® SeqDTR™. DNA selectively binds to the Mag-Bind® SeqDTR™ 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.

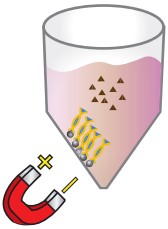
New in this edition:

- The manual has been updated to reflect the product name change from the old name of Mag-Bind® SE DTR to the new name of Mag-Bind® SeqDTR™.

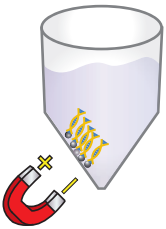
Illustrated Protocol



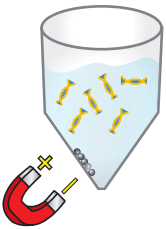
Add Mag-Bind® SeqDTR™ and 85% Ethanol, Mix



Magnetize and remove cleared supernatant



Wash 2 x with 85% Ethanol



Elute and transfer DNA to a new plate

Kit Contents and Storage

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

* *Based on a typical 10 µL reaction volume in a 96-well format*
** *Based on a typical 5 µL reaction volume in a 384-well format*

Storage and Stability

Mag-Bind® SeqDTR™ is stable for at least 9 months from the date of purchase when stored at 2-8°C. **Contents of the kit should never be frozen at any time.**

Mag-Bind® SeqDTR™ 96 Plate Format Protocol

Materials and Equipment to be Supplied by User:

- 85% ethanol (do not use denatured ethanol)
- Magnetic separation device compatible with 96-well PCR plates
- Multichannel pipet
- Reservoirs
- 96-well plate capable of being used in sequencers
- Elution Buffer (Cat# PDR048 or 10 mM Tris pH 8.5, TE Buffer, 0.1 mM EDTA, or diH_2O)

1. Thoroughly shake the Mag-Bind® SeqDTR™ to fully resuspend the magnetic beads.

2. Add 10 µL Mag-Bind® SeqDTR™ to each well.

   Note: Use 10 µL Mag-Bind® SeqDTR™ regardless of the volume of the sequencing reaction.

3. Add 85% ethanol 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 plate on a magnetic separation device to magnetize the Mag-Bind® SeqDTR™. Let sit at room temperature until the Mag-Bind® SeqDTR™ is completely cleared from solution.

5. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® SeqDTR™.

6. Add 100 µL 85% ethanol to each well. It is not necessary to resuspend the Mag-Bind® SeqDTR™.

7. Let sit at room temperature until the Mag-Bind® SeqDTR™ is completely cleared from solution.

8. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® SeqDTR™.

9. Repeat Steps 6-8 for a second 85% ethanol wash step.

10. Leave the plate on the magnetic separation device for 10-15 minutes to air dry the Mag-Bind® SeqDTR™. Remove any residue liquid with a pipettor.

    Note: It is important to dry the Mag-Bind® SeqDTR™ before elution. Residual ethanol may interfere with downstream applications.

11. Add 40 µL Elution Buffer (or 10 mM Tris pH 8.5, TE Buffer, 0.1 mM EDTA, or diH_2O) to each well.

12. Pipet up and down 20 times to mix thoroughly.

13. Let sit at room temperature for 5 minutes.

14. Place the plate on a magnetic separation device to magnetize the Mag-Bind® SeqDTR™. Let sit at room temperature until the Mag-Bind® SeqDTR™ is completely cleared from solution.

15. Transfer 30-35 µL cleared supernatant containing purified sequencing product to a new plate capable of being used in sequencer.

Mag-Bind® SeqDTR™ - 384-well Plate Protocol

Additional Materials Supplied by User:

- 85% ethanol (do not use denatured ethanol)
- Magnetic separation device compatible with 384-well microplates
- Multichannel pipet
- Reservoirs
- 384-well plate capable of being used in sequencers
- Elution Buffer (Cat# PDR048 or 10 mM Tris pH 8.5, TE Buffer, 0.1 mM EDTA, or diH_2O)

1. Thoroughly shake the Mag-Bind® SeqDTR™ to fully resuspend the magnetic beads.

2. Add 5 µL Mag-Bind® SeqDTR™ to each well.

   Note: Use 5 µL Mag-Bind® SeqDTR™ regardless of the volume of the sequencing reaction.

3. Add 85% ethanol 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 plate on a magnetic separation device to magnetize the Mag-Bind® SeqDTR™. Let sit at room temperature until the Mag-Bind® SeqDTR™ is completely cleared from solution.

5. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® SeqDTR™.

6. Add 30 µL 85% ethanol to each well. It is not necessary to resuspend the Mag-Bind® SeqDTR™.

7. Let sit at room temperature until the Mag-Bind® SeqDTR™ is completely cleared from solution.

8. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® SeqDTR™.

9. Repeat Steps 6-8 for a second 85% ethanol wash step.

10. Leave the plate on the magnetic separation device for 10-15 minutes to air dry the Mag-Bind® SeqDTR™. Remove any residue liquid with a pipettor.

    Note: It is important to dry the Mag-Bind® SeqDTR™ before elution. Residual ethanol may interfere with downstream applications.

11. Add 15-20 µL Elution Buffer (or 10 mM Tris pH 8.5, TE Buffer, 0.1 mM EDTA, or diH_2O) to each well.

12. Pipet up and down 20 times to mix thoroughly.

13. Let sit at room temperature for 5 minutes.

14. Place the plate on a magnetic separation device to magnetize the Mag-Bind® SeqDTR™. Let sit at room temperature until the Mag-Bind® SeqDTR™ is completely cleared from solution.

15. Transfer the cleared supernatant containing purified sequencing product to a new plate capable of being used in sequencer.

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 **800-832-8896.**

Possible Problems and Suggestions

Problem	Cause	Solution
Dye terminator remain in the eluted DNA and caused blobs	Supernatant is not removed completely	Make sure to remove any liquid drops from each well of the plate.
	Too much BigDye®	Use less BigDye® per reaction.
	Insufficient washing	During steps 6-9, mix beads to wash more effectively.
Problem	Cause	Solution
Low Signal	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.
	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)

Product	Part Number
Nuclease-free Water (1 mL)	PD092
Elution Buffer (100 mL)	PDR048
Multichannel Disposable Reservoirs (100/pk)	AC1331-01
96-well Microplate (500 µL) (25/pk)	EZ9604-02
Mag-Bind® SeqDTR™ (50 mL)	M1300-08
Mag-Bind® SeqDTR™ (500 mL)	M1300-50
Mag-Bind® E-Z Pure (50 mL)	M1380-01
Mag-Bind® E-Z Pure (500 mL)	M1380-02