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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 Dye-Terminator Removal)** is designed to effective and reliable removal of unincorporated terminators from sequencing reaction. 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, nontargeted 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.

Storage and Stability

Mag-Bind® SE DTR are stable for at least 9 months from the date of purchase when stored at 5°C-8°C. **Contents of the kit should not be frozen at any time.**

Kit Components

Product NO.	M1300-05	M1300-08	M1300-50
Purification	500/1000*	5000/10,000*	50,000/100,000*
Mag-Bind® SE DTR	5 ml	50ml	500 ml
Manual	1	1	1

* 96 or 384 well format

Additional Materials Supplied by User:

- 85% ethanol
- Magnetic Separation Device
- Multichannel pipet
- Polypropylene reservoirs
- 96-well processing plate (Note: the type of collection plate to be used depends on the type of Magnetic Separation Stand used. For OBI's MSTND-01, a 500 µl round bottom plate is recommended (Cat #EZ9604)

Mag-Bind® SE DTR 96 Sequencing Dye Removal Protocol

1. **Gently 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 10µl of Mag-Bind® SE DTR regardless of the volume of the sequencing reaction.

3. **Add either 85% ethanol or absolute ethanol according to table below and mix the sample throughly by pipetting up and down 7-10 times.** Note: If absolute ethanol is used, make sure it is not denatured ethanol.

Reaction Volume (µl)	85% ethanol (µl)	100% ethanol
5	30	26
10	40	36
15	50	43
20	60	51

4. **Place the sample plate on a magnetic separation device for 5-7 minutes or until the solution clears.** If using Omega Bio-Tek's MSD-01 magnet, the magnetic beads will form a pellet at corner of each well adjacent to the magnet.

5. **Aspirate and discard the cleared supernatant.**

6. **With the plate on the magnet, add 100µl of 85% ethanol to each well, wait 2-3 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. Add another 100µl 85% ethanol and incubate at room temperature for 1-2 minutes.**

8. **Aspirate and discard the supernatant.**

9. **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 (0.1M EDTA or Di H_2O).** Mix throughly by pipetting up and down for 20 times.

11. Incubate at room temperature for 15-20 minutes.

12. **Place the plate onto a magnetic separation device and wait 7-10 minutes or until the magnetic beads are cleared from solution.**

13. **Transfer 20 µl of the cleared supernatant** contains purified sequencing product into a new microplate.

Mag-Bind® SE DTR 384 Sequencing Dye Removal Protocol

1. **Gently shake the Mag-Bind® SE DTR bottle to fully resuspend the magnetic beads.**

2. **Add 5 µl of Mag-Bind® SE DTR to each sample.**
   Note: Use 5 µl Mag-Bind® SE DTR for 384-well plate regardless the volume of the sequencing reaction.

3. **Add either 85% ethanol or absolute ethanol according to table below and mix the sample throughly by pipetting up and down 7-10 times.** Note: If absolute ethanol is used, make sure it is not denatured ethanol.

Reaction Volume (µl)	85% ethanol (µl)	100% ethanol
5	30	26
10	40	36
15	50	43

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

5. Aspirate and discard the cleared supernatant.

6. **With the plate on the magnet, add 30 µl of 85% ethanol to each well, wait 2-3 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. Add another 30µl 85% ethanol and incubate at room temperature for 1-2 minutes .**

8. Aspirate and discard the supernatant.

9. 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 (0.1M EDTA or Di H_2O).** Mix throughly by pipetting up and down for 20 times.
11. Incubate at room temperature for 15-20 minutes.

12. Place the plate onto a magnetic separation device and wait 7-10 minutes or until the magnetic beads are cleared from solution.

13. **Transfer 10 µl of the cleared supernatant contains purified sequencing product into a new microplate.**

Trouble shooting Guide

Problem	Likely Cause	Suggestions
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
	Ethanol concentration is not correct	Make sure to use correct volume of ethanol
Low signal intensity	Insufficience mix of the magnetic beads	Ensure the magnetic beads are fully resuspended before use.
	Magnetic beads are lost during the process	Make sure not to remove any magnetic beads during aspiration.
	Low ethanol concentration	Check the ethanol concentration, use fresh ethanol if necessary

For technical support or to place orders, contact Omega Bio-Tek:
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