Contents

Introduction

The Mag-Bind® Oligonucleotide Kit allows rapid and reliable isolation of DNA fragments from enzyme reactions such as PCR and restriction enzyme reaction. This kit can recover DNA fragments as low as 10 bp. The system combines Omega Bio-Tek's proprietary chemistries with the reversible nucleic acid-binding properties of paramagnetic beads to eliminate excess nucleotides, salts and other contaminants. This kit is designed for both manual and fully automated processing. Purified DNA fragments can be used for microarrays, automated fluorescent DNA sequencing, restriction enzyme digestion and other applications.

Overview

The Mag-Bind® paramagnetic particles technology provides a better solution for nucleic acid purification than centrifugation and vacuum based technologies. It can be easily scaled up and it provides very user-friendly handling procedures. If using the Mag-Bind® Oligonucleotide Kit for the first time, please read this booklet to become familiar with the procedures. DNA samples are first mixed with the paramagnetic particles, to which DNA selectively binds. With just two rapid wash steps, trace contaminants such as nucleotides, salt and enzymes are removed. Pure DNA is eluted in Elution Buffer or water. Purified DNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

All components of the Mag-Bind® Oligonucleotide Kit are stable for at least 24 months from date of purchase when stored at 22°C-25°C.

Kit Contents

Product Number	M2514-00	M2514-01	M2514-02
Purifications	5	50	200
Mag-Bind® Particles Solution	55 µL	550 µL	2.1 mL
MGC Binding Buffer	2 mL	12 mL	4 x 12 mL
LPA Buffer	55 µL	550 µl	2.1 mL
SPW Wash Buffer	2 mL	15 mL	2 x 25 mL
Elution Buffer	2 mL	10mL	30 mL
Instruction Manual	1	1	1

Before Starting

Please read this entire booklet to become familiar with the Mag-Bind® Oligonucleotride Kit procedures.

- Dilute **SPW Wash Buffer** Concentrate with **ethanol** as follows and **store at room temperature**.

M2514-00	Add 8 ml absolute (96%-100%) ethanol.
M2514-01	Add 60 ml absolute (96%-100%) ethanol to each bottle.
M2514-02	Add 100mL absolute (96%-100%) ethanol to each bottle.

- Dilute **MGC Binding Buffer** with **ethanol** as follows and **store at room temperature**:

.

M2514-00	Add 8 ml absolute ethanol (96%-100%)
M2514-01	Add 48ml absolute ethanol (96%-100%)
M2514-02	Add 48ml absolute ethanol (96%-100%) to each bottle

Protocol for Small DNA Fragments(≤150 bp)

Material to be Supplied by User

- 1.5 mL Centrifuge Tube
- Absolute ethanol (96%-100%)
- 80% ethanol (*for small fragments protocol only*)
- Magnetic Separation Stand (Cat #MSD-02)

DNA fragments less than 150 bp can be successfully purified by following this protocol.

1. Place the centrifuge tube containing DNA samples on the bench and measure the volume of the sample.

2. Add 10 µl of LPA Buffer followed by 10 µl of Mag-Bind® Particles Solution to each sample to each sample.

   Note: The Mag-Bind® particles tend to settle and bead together. The particles must be fully resuspended by shaking or vortexing before use.

3. Add 4 volumes of MGC Binding Buffer diluted with ethanol to each sample.

4. Mix each sample by pipetting up and down 4-5 times, then incubate 1 minute at room temperature. Mix again by pipetting up and down 4-5 times.

5. Place the Centrifuge tube onto a magnetic separation stand to magnetize the Mag-Bind® particles. Solution will clear when particles have completely migrated toward the magnets.

6. Remove and discard the cleared supernatant.

7. Remove the centrifuge tube containing the Mag-Bind® particles from the magnetic separation stand. Add 400 µl of SPW Wash Buffer diluted with ethanol to each well.

8. Mix each sample by pipetting up and down 4-5 times. Incubate 1 min at room temperature. Mix again by pipetting up and down 4-5 times.

9. Place the tube onto the magnetic separation stand to magnetize the Mag-Bind® particles.

10. Add 400 µl of 80% ethanol to the tube. Mix each sample by pipetting up and down 4-5 times. Incubate 1 min at room temperature. Mix again by pipetting up and down 4-5 times

11. Place the tube onto the magnetic separation stand to magnetize the Mag-Bind® particles

12. Allow the samples to dry on the magnetic separation stand for 5-10 minutes. Remove any liquid residue from the wells by pipetting.

    Note: Complete drying of samples is critical for removal of ethanol that might otherwise interfere with downstream applications. Avoid over-drying the samples, however, which will make resuspension and elution difficult in the following steps.

13. After the tube has dried completely, remove it from the magnetic separation stand and add 50-100 ul Elution Buffer or water to each centrifuge tube to elute DNA from the magnetic particles.

14. Mix each sample by pipetting up and down 4-5 times. Incubate 1 minute at room temperature. Mix again by pipetting up and down 4-5 times.

15. Place the tube onto the magnetic separation stand to magnetize the magnetic particles.

16. Transfer the cleared supernatant containing purified DNA to a 1.5 mL centrifuge tube.

17. Store the centrifuge tube at 4° C if storage is only for a few days. For long-term storage samples should be kept at -20° C.

Mag-Bind® Oligonucleotride Protocol

Note: This protocol is for DNA fragments larger than 150 bp.

1. Read the manufacturer's instruction manual for the magnetic separation stand, if provided.

2. Place the Centrifuge tube containing DNA samples on the bench and measure the volume of the sample.

3. Add 10 µl Mag-Bind® Particles Solution to each sample.

   Note: The Mag-Bind® particles tend to settle and bead together. The particles must be fully resuspended by shaking or vortexing before use.

4. Add 2 volumes of MGC Binding Buffer diluted with ethanol to each sample.

5. Mix each sample by pipetting up and down 4-5 times, then incubate 1 minute at room temperature. Mix again by pipetting up and down 4-5 times.

6. Place the centrifuge tube onto a magnetic separation stand to magnetize the Mag-Bind® particles. Solution will clear when particles have completely migrated toward the magnets.

7. Remove and discard the cleared supernatant.

8. Remove the centrifuge tube containing the Mag-Bind® particles from the magnetic separation stand. Add 400 µl of SPW Wash Buffer diluted with ethanol to each well.

9. Mix each well by pipetting up and down 4-5 times, then incubate 1 minute at room temperature. Mix again by pipetting up and down 4-5 times.

10. Place the centrifuge tube onto the magnetic separation stand to magnetize the Mag-Bind® particles. Solution will clear when particles have completely migrated toward the magnets.

11. Allow the samples to dry on the magnetic separation stand for 5-10 minutes. Remove any liquid residue from the wells by pipetting.

    Note: Complete drying of samples is critical for removal of ethanol that might otherwise interfere with downstream applications. Avoid over-drying the samples, however, which will make resuspension and elution difficult in the following steps.

12. After the samples have dried completely, remove the centrifuge tube from the magnetic separation stand and add 50-100ul Elution Buffer or water to each centrifuge tube to elute the DNA from the Mag-Bind® particles.

13. Mix each sample by pipetting up and down 4-5 times. Incubate 1 minute at room temperature. Mix again by pipetting up and down 4-5 times.

14. Place the centrifuge tube onto the magnetic separation stand to magnetize the Mag-Bind® particles. Solution will clear when particles have completely migrated toward the magnets.

15. Transfer the cleared supernatant containing purified DNA to a new centrifuge tube.

16. Store the centrifuge tube at 4° C if storage is only for a few days. For long-term storage samples should be kept at -20° C.

Troubleshooting

Problem	Cause	Suggestions
Low yield	Low PCR product yield	Increase the number amplification cycles for PCR
	Smaller PCR product size	Small PCR fragments normally give lower yield. See page 6 for optimized protocol very small fragments
	Ethanol residue	During the drying step, remove any liquid from bottom of the well
	Particle loss during the procedure	Increase magnetization time. Aspirate more slowly
	DNA remains bound to beads	Increase elution volume to 200 µl
	Incompletely resuspension of the beads during elution	Fully suspend the beads by pipetting up and down.
Primer carryover	Insufficient wash of the particles	Wash the beads one more time with MGW Wash Buffer
Non-specific amplification products were not removed	The size of the non-specific amplification products are larger than 100bp.	Non-specific amplification products larger than 100bp are not efficiently removed from PCR products.
Problems in downstream applications	Salt carry-over.	Wash Buffer must be at room temperature.
	Ethanol carry-over	Ensure the beads are completely dried before elution