E.Z.N.A.® Mag-Bind ® Dye Terminator Removal Kit

Product M1320

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. The E.Z.N.A.® Mag-Bind™ Dye-Removal Kit is designed for effective and reliable removal of unincorporated terminators from sequencing reactions

Magnetic particles offer greater flexibility than centrifugation- and vacuum-based formats for nucleic acid purification. Among these benefits are scalability, easier handling and in-solution kinetics. The purification process results in highly purified sequencing products.

Benefits

The E.Z.N.A.® Mag-Bind™ Dye terminator Removal Kit offers:

- **Fast** - less than 30 minutes to recover pure DNA from a standard sequencing reaction
- **Convenient** - Fully automated protocol
- **Reliable** - giving consistent recovery at high quality

Kit Contents

Product Number	**M1320-00**	**M1320-01**	**M1320-02**
Isolations	1 x 96	4 x 96	24 x 96
Mag-Bind™ Particle Solution E	1 ml	4 ml	24 ml
MPG Wash Buffer	3 ml	15 ml	75 ml
Instruction Manual	1	1	1

Storage and Stability

All components of the E.Z.N.A.® Mag-Bind™ Dye-Removal Kit are stable for at least 24 months from the date of purchase when stored at 22°C-25°C.

Material to Be Provided by User

- Multiple-channel pipettor
- Absolute (96%-100%) ethanol
- 96-well microplate
- Magnetic Separation Devices
- Molecular biology grade water

Before Starting

IMPORTANT	MPG Wash Buffer must be diluted with absolute ethanol before use: M1320-00: Add 7 ml absolute ethanol to each bottle M1320-01: Add 35 ml absolute ethanol to each bottle M1320-02: Add 175 ml absolute ethanol to each bottle

Please take a few minutes to read this booklet thoroughly and become familiar with the protocol. Prepare all materials required before starting the procedure.

- **Prepare the Mag-Bind™ particle by adding absolute ethanol (96-100%). Mix throughly before use.**

IMPORTANT	**Mag-Bind™ Particle Solution E must be diluted with absolute ethanol before use:** **M1320-00: Add 4 ml absolute ethanol to each bottle** **M1320-01: Add 16ml absolute ethanol to each bottle** **M1320-02: Add 96ml absolute ethanol to each bottle**

The E.Z.N.A.® Mag-Bind™ Dye terminator Removal Procedure

1. Determine the volume of the crude sequencing product and transfer the sample into 96-well microplate. **Add 10 µl molecular grade water to each sample.**

2. Add **2 volume** of Mag-Bind® Particle/ethanol mixture and mix thoroughly by pipetting.

3. Incubate 10 minutes at room temperature. Place the microplate on a magnetic separation device designed for 96-well plate. Wait for 5 minute or until the solution is clear of beads.

4. Aspirate entire solution with multiple channel pipettor. Do not disturb the Mag-Bind™ Particle pellet.

5. Dispense 100µl MPG Wash Buffer into each well, resuspend Mag-Bind Particle pellet by pipetting. Incubate for 1 minute at room temperature.

6. Place the microplate on a magnetic separation device, and wait 1 minute or until the solution is clear of beads.

7. Aspirate entire solution with a multiple channel pipettor. Do not disturb the Mag-Bind™ Particle pellet.

8. Dry the Mag-Bind™ Particle pellet by air for 10 minutes.

9. Remove the plate from separation device. Add 15µl - 30µl water to each sample and resuspend Mag-Bind® Particle pellet by pipetting.

10. Incubate 10 minutes at room temperature. Place the microplate on a magnetic separation device designed for 96-well plate. Wait for 1 minute or until the solution is clear of beads.

11. Transfer the cleared supernatant to a new microplate (not provided).

Trouble Shooting Guide

Problem	Cause	Suggestions
Lower signal strength	Inefficient capture of sequencing product	Increase the time of binding step
	Lower binding efficiency.	Add MGT Buffer as instructed in this manual
	Losing magnetic particles during process	Slow aspiration speed increase the magnetizing time Ensure that the pipette tips are offset from the particle pellet
	DNA remains bound to beads	Increase elution volume or increase incubation time for elution
	Incompletely resuspension of the beads during elution	Fully suspend the beads by pipetting up and down.
Strong background signal	Insufficient wash of the particles	Wash the beads one more time with MPG Wash Buffer.
	Sequencing dye bind to the magnetic beads	Reduce or do not add MGT Buffer for Mag-Bind™ Particle Solution
Dye blobs present	inefficient remove of binding/wash solution	Ensure that the supernatant is removed after the beads capture step
	Ethanol carry-over	ensure the beads are completely dried before elution

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