Contents

Introduction

The E.Z.N.A.® Mag-Binds® Forensic DNA Kit allows rapid and reliable isolation of high-quality genomic DNA from Forensic. The Kit allows single or multiple simultaneous processing of samples in under 1 hours. There is no need for phenol/chloroform extraction, and time-consuming steps such as CsCl gradient ultracentrifugation, and precipitation with isopropanol or ethanol, are eliminated. DNA purified using the E.Z.N.A.® Mag-Binds® Forensic DNA Kit is ready for applications such as PCR, Southern blotting and restriction digestion.

Overview

If using the E.Z.N.A.® Mag-Binds® Forensic DNA Kit for the first time, please read this booklet in its entirety to become familiar with the procedures. Forensic samples lysed in a specially formulated buffer. The binding conditions are adjusted so that genomic DNA will selectively bind to the Mag-Binds®. Two rapid wash steps remove trace contaminants such as residual polysaccharides, and pure DNA is eluted in Elution Buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

Most components of the E.Z.N.A.® Mag-Binds® Forensic DNA Kit, except RNase A and OB Protease are stable for at least 24 months from date of purchase when stored at 22°C-25°C. Mag-Bind® Particles Solution B should be stored at 4° C for long-term use. Store RNase A at -20°C. OB Protease store at 15-25°C. During shipment or storage in cool ambient conditions, precipitates may form in Buffer MBL. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Kit Contents

Product Number	M6225-00	M6225-01	M6225-02
Purifications Times	5 Preps	50 Preps	200 Preps
MagSi Particles	120µl	1.2ml	4x1.1 ml
Buffer TL	5 ml	20 ml	60 ml
Buffer MSL	5 ml	20 ml	60 ml
Buffer MP	1.0 ml	20 ml	40 ml
OB Protease	150µl	1.2 ml	4x 1.2 ml
RNase A	12µl	120µl	440µl
Elution Buffer	1ml	20 ml	60 ml
Instruction Booklet	1	1	1

Before Starting

- Please read this booklet thoroughly to become familiar with the E.Z.N.A.® Mag-Bind® Forensic DNA Kit procedures.

- Equilibrate Elution Buffer (or sterile dH_2O water) at 65°C.

- Dilute Buffer MP with absolute ethanol as follows and **store at room temperature**.

M6225-00	Add 6 ml absolute (96%-100%) ethanol.
M6225-01	Add 30 ml absolute (96%-100%) ethanol
M6225-02	Add 60 ml absolute (96%-100%) ethanol

E.Z.N.A.® Mag-Bind® Forensic DNA Kit Magnetic Protocol

Materials to be provided by user:

- Centrifuge capable of 12,000 x g.
- Nuclease-free 15 ml centrifuge tube.
- Water bath preset at 65°C
- Absolute (96%-100%) ethanol
- Magnetic separation strand
- 70% ethanol

1. **Transfer the forensic sample to a sterile microcentrifuge tube and add 180 µl Buffer TL, 20 µl OB Protease.** Vortex to mix well, making sure that the sample is completely immersed in the Buffer TL.

2. Incubate the sample for 1-3 hours at 55°C until lysis is complete.

3. **If RNA-free genomic DNA is required, add 5 µl RNase A to each sample.**

4. **Remove the sample with disposable tweezers or transfer 200 µl the lysate (supernatant) to a new tube.**

5. **Add200 µl Buffer MLS, 20 µl MagSi Particles and 300 ul absolute ethanol (room temperature, 96-100%) to the lysate.** Vortexing/Shaking for 2 minutes or pipetting up and down 20-30 times to mix well.

   Note: Buffer MSL, MagSi Particles and absolute ethanol can be premixed.

6. Place the tube on a magnetic separation device suitable for 1.5 ml tube to magnetize the Mag-Bind® particles .

7. Remove and discard the cleared supernatant.

   Tip: To ensure that all traces of the medium are removed, let the tube sit 2 min at room temperature and remove the remaining liquid by pipettor or invert the tube on paper.

8. Remove the tube containing the Mag-Bind® particles from the magnetic separation device. **Add 400µl Buffer MP3 diluted with ethanol into the tube.**

   Note: Buffer MP3 must be diluted with absolute ethanol (96-100 %) before use in this protocol.

9. Resuspend Mag-Bind® particles pellet by votexing/shaking for 1 minutes or pipetting up and down 20 times.

10. Place the plate onto a magnetic separation device to magnetize the Mag-Bind® particles.Remove and discard the cleared supernatant.

11. Remove the tube containing the Mag-Bind® particles from the magnetic separation device. **Add 500µl of 70% ethanol into the tube.**

12. Resuspend Mag-Bind® particles pellet by vortexing/shaking for 1 minute or pipetting up and down 20 times.

13. Place the plate onto a magnetic separation device to magnetize the Mag-Bind® particles.Remove and discard the cleared supernatant.

14. Remove the tube containing the Mag-Bind® particles from the magnetic separation device. **Add 500µl of 70% ethanol into the tube.**

15. Resuspend Mag-Bind® particles pellet by vortexing/shaking for 1 minute or pipetting up and down 20 times.

16. Place the plate onto a magnetic separation device to magnetize the Mag-Bind® particles.Remove and discard the cleared supernatant.

17. Leave the tube to air dry on the magnetic separation device for 5 minutes. Remove any residue liquid from tube by pipetting.

18. **Remove the tube from magnetic separation device. Add 50-100ul Elution Buffer or water to elute DNA from the magnetic particles.**

19. Resuspend Mag-Bind® particles by vortexing/shaking or pipetting up and down 20 times. Incubate 5-10 minutes at room temperature. Repeating the mix by vortexing or pipetting up and down for 1 minutes.

20. Place the tube onto a magnetic separation device to magnetize the Mag-Bead® particles.

21. Transfer the cleared supernatant containing purified DNA to a new 1.5 ml tube.

E.Z.N.A.® Mag-Bind® Forensic DNA Kit Spin Protocol

The E.Z.N.A®. Mag-Binds® Forensic DNA Kit May be proceed by Centrifugation if without magnetic strand available. The purity of genomic DNA isolated by Spin Protocol may be low than Magnetic protocol because some unsoluble particle precipitated with genomic DNA, while only genomic DNA can be collected in Magnetic Protocol. In most specimens, genomic DNA isolated by this protocol can be used in PCR, Southern blot.

1. **Transfer the forensic sample to a sterile microcentrifuge tube and add 180 µl Buffer TL, 20 µl OB Protease.** Vortex to mix well, making sure that the sample is completely immersed in the Buffer TL.

2. Incubate the sample for at least 1 hours at 55°C until lysis is complete.

3. Remove the Forensic sample with disposable tweezers or Centrifuge at 15,000 x g for 5 min and transfer 200ul supernatant to a new tube.

4. **Add200 µl Buffer MLS, 20 µl MagSi Particles and 300 ul absolute ethanol (room temperature, 96-100%) to the lysate.** Vortexing/Shaking for 2 minutes or pipetting up and down 20-30 times to mix well.

   Note: Buffer MSL, MagSi Particles and absolute ethanol can be premixed.

5. Centrifuge at 10,000 x g for 1 min. Remove and discard the cleared supernatant.

   Tip: To ensure that all traces of the solution are removed, invert the tube on paper for 2 min.

6. **Add 400 ul of Buffer MP3 and vortex to resuspend Mag-Bind® Pellets**.

   Note: Buffer MP3 should not diluted with absolute ethanol before use in this spin protocol.

7. **Add 500 ul of 70% ethanol, vortex to mix.**

8. Centrifuge at 10,000 x g for 1 min. Remove and discard the cleared supernatant.

9. Leave the tube to air dry on the magnetic separation device for 5 minutes.

10. **Add 50-100ul Elution Buffer or water to elute DNA from the magnetic particles.**

11. Resuspend Mag-Bind® particles by vortexing. Incubate 5-10 minutes at room temperature.

12. Centrifuge at 10,000 x g for 1 min and transfer the supernatant into a new tube. Store Purified DNA at -20°C.

Yield and quality of DNA

Determine the absorbance of an appropriate dilution (20- to 50-fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

$$DNA\ concentration = Absorbance_{260} \times 50 \times (Dilution\ Factor)\ \mu g/ml$$

The ratio of $(absorbance_{260})/(absorbance_{280})$ is an indication of nucleic acid purity. A value greater than 1.8 indicates > 90% nucleic acid. Alternatively, quantity (as well as quality) can sometimes best be determined by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations.

Troubleshooting Guide

Problem	Cause	Suggestions
Low DNA yield	Incomplete disruption of starting material.	For both dry and fresh samples, obtain a fine homogeneous powder before adding Buffer SP1.
	Poor lysis of tissue.	Decrease amount of starting material or increase amount of Buffers SP1 and SP2.
	Loss the MagBeads® particle during operation	Carefully avoid remove the MagBeads® particles during aspiration
	DNA remains bound to MagBeads® Particles	Increase elution volume and incubate on column at 65ºC for 5 min elution
	DNA washed off.	Dilute MGB Binding Buffer and SPM Wash Buffer by adding appropriate volume of absolute ethanol prior to use (page 3).
Problems in downstream applications	Salt carry-over.	Wash Buffer must be at room temperature.
	Ethanol carry-over	Dry the MagBeads® particle before elution.