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Introduction

The E.Z.N.A.® Mag-Bind Forensic DNA Isolation Kit is designed to provide a rapid and easy method for the isolation of genomic DNA from forensic samples such as dry blood, buccal swabs, and sperm for consistent PCR and Southern analysis. This kit can also be used for the preparation of genomic DNA from mouse tail snips, whole blood, buffy coat, serum, and plasma. The kit allows single or multiple, simultaneous processing of samples. High quality genomic DNA isolated with Mag-Bind technology is suitable for direct use in most downstream applications such as amplifications and enzymatic reactions. This system can be easily adapted with automated system and the procedure can be scaled up or down, allowing purification from various amounts of starting materials.

Principle

E.Z.N.A.® Mag-Bind Forensic DNA Isolation Kits use the reversible binding properties of the Mag-Bind ® paramagnetic particles to provide a fast and flexible method for isolating genomic DNA from different forensic sources. Samples are first lysed with a specially formulated buffer containing detergent in the presence of Proteinase K. After adjust the binding condition, the sample was mixed with Mag-Bind particles and the genomic DNA was bound to the surface of Mag-Bind magnetic particles. Proteins, polysaccharides, and cellular debris are efficiently washed away with few wash steps. Pure DNA is then eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

All components of the E.Z.N.A.® Mag-Bind Forensic DNA Isolation Kit, except the Mag bind Particles and Binding Enhancer can be stored at 22°C-25°C. Proteinase K can be stored at room temperature. For long-term storage (>12 months), store Proteinase K at 2-8 °C. Mag Bind Particles and Binding Enhancer should be stored at 2-8 °C, Under these conditions, performance of all components of the kit are guaranteed at least 12 months. Under cool ambient conditions, a precipitate may form in the Buffer TL and MSL. In case of such an event, heat the bottle at 50°C to dissolve the precipitate.

Kit Contents

Product	M6225-00	M6225-01	M6225-02
Purification	5	50	200
Mag-Bind Particles C	55 µL	530 µL	2.2 mL
Buffer MSL	1.5 mL	15 mL	60 mL
Buffer TL	1.5 mL	15 mL	60 mL
SPM Buffer	2 mL	12 mL	50 mL
Binding Enhancer	55 µL	550 µL	2.2 mL
MP Buffer	2 mL	20 mL	40 mL
Elution Buffer	2 mL	30 mL	2 x 50 mL
Proteinase K Solution	150 µL	1.5 mL	6 mL
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Product	M1427-00	M1427-01	M1427-02
Purification	1 x 96	4 x 96	20 x 96
Mag-Bind Particles C	1.1 mL	4.2 mL	21 mL
Buffer MSL	25 mL	100 mL	500 mL
Buffer TL	25 mL	100 mL	500 mL
SPM Buffer	25 mL	100 mL	2 x 200 mL
MP Buffer	20 mL	80 mL	400 mL
Elution Buffer	15 mL	30 mL	2 x 50 mL
Binding Enhancer	1.1 mL	4.4 mL	22 mL
Proteinase K Solution	3 mL	12 mL	60 mL
User Manual	1	1	1

CAUTION! Buffer MSL contains a chaotropic salt. Please wear gloves, and appropriate eye ware while performing this procedure.

NOTE: The E.Z.N.A.® Mag-Bind Forensic DNA Isolation Kit is supplied with enough buffer for the standard protocol. However, due to increased volumes called for in some protocols, fewer preparations may be performed. Also, additional buffers can be purchased separately from Omega Bio-Tek. See the Accessories section in the catalog or call customer service for price information

Important	SPM Buffer must be diluted with absolute ethanol as follows **M6225-00** Add 8 mL ethanol **M6225-01** Add 48 mL ethanol / bottle **M6225-02** Add 200 ml ethanol/bottle **M1427-00** Add 100 mL ethanol / bottle **M1427-01** Add 400 ml ethanol/bottle **M1427-02** Add 800 mL ethanol / bottle
	Prepare **FRESH** Buffer MP/Ethanol as follows. This mixture can only be stored at room temperature for two weeks. **M6225-00** Add 3 ml absolute ethanol **M6225-01** Add 30 ml absolute ethanol **M6225-02** Add 60 ml absolute ethanol **M1427-00** Add 30 ml absolute ethanol **M1427-01** Add 120 ml absolute ethanol **M1427-02** Add 600 ml absolute ethanol

New in this edition:

- Proteinase K is now supplied in a liquid form eliminating the step to resuspend prior to use. Proteinase K Solution can also be stored at room temperature for 12 months.
- Proteinase Storage Buffer is no longer included in this kit.

Preparing Reagents

Forensic DNA Protocol

Protocol For Isolation of DNA From Dried Blood, Body Fluids and Sperm Spots

Dried blood, body fluids, and sperm samples on filter paper can be processed using the following method. This kit can also be used for samples collected by using other specimen collection papers

User Supplied Materials:

Centrifuge capable of 13,000 x g or 3,000 x g for 96 well plates
Isopropanol
96 well magnetic stand or 1.5 mL magnetic stand
1.5 ml tube or 500 µL microplate
Absolute Ethanol (96-100%).
An incubator capable of 70 °C
Deep Well of 1.2 mL round well plate
Sealing film or caps for Deep well or 1.2 mL round well plate

Before Starting:

Set an Incubator 55 °C after step 2 set to 60°C
Set an Incubator to 70 °C
Prepare Reagents according to preparing reagents section

1. Cut or punch out the blood spot (or other sample) from the filter paper. (Up to 200 µL of blood can be used for each spot.) Tear or cut filter into small pieces and place into a microfuge tube or 96 well plate. A deep well or 1.2 mL round well plate can be used.. Note: Use 3-4 punched cycles (3mm diameter) for each DNA isolation.

2. Add 200ul Buffer TL and 25 ul Proteinase K Solution and mix by vortexing. Seal the plate with sealing film or caps. Incubate for 30-45 minutes at 55°C with occasional mixing.

3. Add 200ul Buffer BL and incubate at 70°C for 10 minutes. Vortex every 2 min to mix Seal the plate with sealing film or caps.

4. Centrifuge at maximum speed (13,000 - 20,000 x g) for 5 minutes or 3,000 x g for 10 minutes fo r96 well plates.

   NOTE: If maximum DNA recovery is required, the well of 96-well lysate clearance plate can be used to collect maximum volume of the liquid.

5. Transfer 400 µl (for single tube) or 200µl (for 96-well plate) of lysate from previous step to a 1.5 ml tube or 96-well microplate (500ul).

6. Add 280 µl or 140 µl of isopropanol followed by 10µl of Mag-Bind particles and mix throughly by vortexing or pipetting up and down for 20 times.

7. Incubate at room temperature for 5 minutes.

8. Place the tube or plate to a magnetic separation device suitable for 1.5 ml tube or 96-well microplate to magnetize the Mag-Bind particles. The solution should be cleared after all the magnetic beads are pelleted.
9. Carefully remove and discard the cleared supernatant by pipetting.

10. Remove the tube or plate containing the Mag-Bind particles from the magnetic separation device.

11. Add 400 µL or 300 µL MP Buffer to each sample and mix throughly by vortexing or pipetting up and down for 20 times.

    Note: It is critical to wash the magnetic particles by breaking up the magnetic particle pellet for DNA purity.

12. Place the tube or plate to a magnetic separation device suitable for 1.5 ml tube or 96-well microplate to magnetize the Mag-Bind particles.

13. Carefully remove and discard the cleared supernatant by pipetting.

14. Remove the tube or plate containing the Mag-Bind particles from the magnetic separation device.

15. Add 500 µL or 300 µL SPM Buffer to each sample and mix throughly by vortexing or pipetting up and down for 20 times.

    Note: SPM Buffer must be diluted with ethanol before use.

16. Place the tube or plate to a magnetic separation device suitable for 1.5 ml tube or 96-well microplate to magnetize the Mag-Bind particles.

17. Carefully remove and discard the cleared supernatant by pipetting.

18. Remove the tube or plate containing the Mag-Bind particles from the magnetic separation device.

19. Wash the Mag-Bind particles again by repeating step 16-18 with SPM Buffer.

20. Leave the tube to air dry on the magnetic separation device for 5-10 minutes. Remove any residue liquid from tube by pipetting.

21. Remove the tube or plate containing the Mag-Bind particles from the magnetic separation device.

22. Add 50-200ul of Elution Buffer to the tube or each well of the microplate. Mix throughly by vortexing or pipetting up and down for 50 times.

23. Incubate at 60°C for 15 minutes.

24. Place the tube or plate to a magnetic separation device suitable for 1.5 ml tube or 96-well microplate to magnetize the Mag-Bind particles.

25. Carefully transfer the cleared supernatant contains eluted DNA to a clean 1.5 ml tube or microplate.

Protocol For Isolation of Genomic DNA From Sperm

This protocol can be used for fresh or frozen semen samples with equal efficiency. Frozen samples must to be thawed thoroughly before use. Note that lysis time will vary depending on the size and density of the source material.

User Supplied Materials:

Centrifuge capable of 13,000 x g
Isopropanol
96 well magnetic stand or 1.5 mL magnetic stand
1.5 ml tube or 500 µL microplate
Absolute Ethanol (96-100%).
An incubator capable of 70 °C

Make the following buffer before starting:

Buffer SL	200 mM NaCI 20mM Tris-HCl, pH 8.0 20mM EDTA, pH 8.0 4% SDS 1% ß-mercaptoethanol

Before Starting:
Set an Incubator 55 °C after step 2 set to 60°C
Set an Incubator to 70 °C
Prepare Reagents according to preparing reagents section

1. Add 100µL of sperm to 100µL of Buffer A in a glass (Corex) centrifuge tube. Vortex for 10 sec at full speed. Only use Corex tubes to prevent attachment of the sperm cells to the tube walls.

2. Add 20 µL Proteinase K Solution (20 mg/mL)and incubate for 2 hours at 60°C. Invert the tube occasionally to disperse the sample or place on a rocking platform.

3. Add 220 µL Buffer MSL to the sample and mix by vortexing.

4. Centrifuge at full (>13,000 x g) for 5 minutes .

5. Transfer **400 µl** sample to a new 1.5 ml tube. For 96-well microplate procedure, transfer **250µl** of sample to each well of the microplate.

6. Add **270µl** (for singe tube) or **170µl** absolute ethanol to each sample.

7. Add **10µl** of Mag-Bind particles and mix throughly by vortexing or pipetting up and down for 20 times.

8. Incubate at room temperature for 5 minutes.
9. Place the tube or plate to a magnetic separation device suitable for 1.5 ml tube or 96-well microplate to magnetize the Mag-Bind particles. The solution should be cleared after all the magnetic beads are pelleted.

10. Carefully remove and discard the cleared supernatant by pipetting.

11. Remove the tube or plate containing the Mag-Bind particles from the magnetic separation device.

12. Add **500 µL** (for 1.5 ml tube) or **300 µL** (for 96-well plate) SPM Buffer to each sample and mix throughly by vortexing or pipetting up and down for 20 times.

13. Place the tube or plate to a magnetic separation device suitable for 1.5 ml tube or 96-well microplate to magnetize the Mag-Bind particles.
14. Carefully remove and discard the cleared supernatant by pipetting.

15. Remove the tube or plate containing the Mag-Bind particles from the magnetic separation device.

16. Wash the Mag-Bind particles again with SPM Buffer by repeating step 11-13.

17. Leave the tube to air dry on the magnetic separation device for 5-10 minutes. Remove any residue liquid from tube by pipetting.

18. Remove the tube or plate containing the Mag-Bind particles from the magnetic separation device.

19. Add 50-200 µl of Elution Buffer to the tube or each well of the microplate. Mix throughly by vortexing or pipetting up and down for 50 times.

20. Incubate at 60°C for 15 minutes.

21. Place the tube or plate to a magnetic separation device suitable for 1.5 ml tube or 96-well microplate to magnetize the Mag-Bind particles.

22. Carefully transfer the cleared supernatant contains eluted DNA to a clean 1.5 ml tube or microplate.

Protocol For Isolation of Genomic DNA From Buccal Swabs:

User Supplied Materials:

Isopropanol
96 well magnetic stand or 1.5 mL magnetic stand
1.5 ml tube or 500 µL microplate
Absolute Ethanol (96-100%).
An incubator capable of 70 °C
Deep Well of 1.2 mL round well plate compatible with MSD-01B
Magnetic Stand MSD-01B
Sealing film or caps for Deep well or 1.2 mL round well plate

Before Starting:

Set an Incubator 55 °C after step 2 set to 60°C
Set an Incubator to 70 °C
Prepare Reagents according to preparing reagents section

This protocol has been tested for the following swab types: cotton, C.E.P. (Life Science). Typical yields from these swabs are 0.5 - 3 µg DNA.

1. Scrape the swabs firmly against the inside of each cheek 6 -7 times. Air or vacuum dry the swabs for 2 hours after collection. The person providing the sample should not eat or drink for at least 30 minutes prior to the sample collection.

2. Carefully break or cut off the end part of the swab or brush into a 1.2 ml plate and add 400 µL TL to the tube. Add 20 µL Proteinase K Solution (20mg/ml). Seal the Plate with Sealing Film. Incubate 60 minutes at 56°C.

3. Transfer 280 µl of lysate into a 1.2 mL Round Well Plate compatible with MSD-01B.

4. Add 280 µL Buffer MSL to the sample. Seal The plate with Sealing Film(not provided) Mix immediately by vortexing for 30 seconds.

5. Remove the Sealing film. Add 380 µl Isopropanol and 10 µl Mag Bind Particles C and 10 µL of binding enhancer.

6. Incubate at room temperature for 5 minutes.

7. Place the tube or plate to a magnetic separation device suitable for 1.5 ml tube or 96-well microplate to magnetize the Mag-Bind particles. The solution should be cleared after all the magnetic beads are pelleted.

8. Carefully remove and discard the cleared supernatant by pipetting.

9. Remove the tube or plate containing the Mag-Bind particles from the magnetic separation device.

10. Add **300 µL** MP Buffer and mix throughly by vortexing or pipetting up and down for 20 times.

11. Place the tube or plate to a magnetic separation device suitable for 1.5 ml tube or 96-well microplate to magnetize the Mag-Bind particles.

12. Carefully remove and discard the cleared supernatant by pipetting.

13. Add 400 µL of SPM Buffer and mix throughly by vortexing or pipetting up and down 20 times.

14. Place the tube or plate to a magnetic separation device suitable for 1.5 ml tube or 96-well microplate to magnetize the Mag-Bind particles.

15. Carefully remove and discard the cleared supernatant by pipetting.

16. Repeat Steps 13-15

17. Leave the tube to air dry on the magnetic separation device for 5-10 minutes. Remove any residue liquid from tube by pipetting.

18. Remove the tube or plate containing the Mag-Bind particles from the magnetic separation device.

19. Add 50-100 µL of Elution Buffer to the tube or each well of the microplate. Mix throughly by vortexing or pipetting up and down for 50 times.

20. Incubate at 60°C for 15 minutes.

21. Place the tube or plate to a magnetic separation device suitable for 1.5 ml tube or 96-well microplate to magnetize the Mag-Bind particles.

22. Carefully transfer the cleared supernatant contains eluted DNA to a clean 1.5 ml tube or microplate.

Protocol for Isolation of Bacterial DNA From Biological Fluids:

1. Pellet bacteria by centrifuging 10 minutes at 8,000rpm.

2. Resuspend bacterial pellet with 200 µL TL buffer.

3. Follow the protocol for dried blood, body fluids and sperm spot (Page 4) from Step 3.

Protocol For Isolation of DNA From Saliva:

1. Collect 200 µL saliva in a 1.5 mL centrifuge tube contains 200 µL Buffer MSL and 20 µl of Proteinase K Solution.

2. Mix the sample throughly by vortexing or pipetting up and down for 20 times.

3. Incubate at 65°C for 30 minutes.

4. Centrifuge at 14,000 x g for 2 minutes and transfer the sample to a new 1.5 ml tube.

5. Optional: If RNA-free DNA is desired, add 10 µl of RNase A (25mg/ml) and incubate at room temperature for 5 minutes.

6. Add 10µl Mag-Bind Particles followed by 290 µl of absolute ethanol. Mix throughly by vortexing or pipetting up and down for 20 times.

7. Incubate at room temperature for 5 minutes.

8. Place the tube or plate to a magnetic separation device suitable for 1.5 ml tube or 96-well microplate to magnetize the Mag-Bind particles. The solution should be cleared after all the magnetic beads are pelleted.

9. Carefully remove and discard the cleared supernatant by pipetting.

10. Remove the tube or plate containing the Mag-Bind particles from the magnetic separation device.

11. Add **300 µL** MP Buffer to each sample and mix throughly by vortexing or pipetting up and down for 20 times.

12. Follow the standard protocol for dried blood or body fluids (Page 5) from Step 13- 26.

Protocol For Isolation of DNA From Hair, Nails and Feathers:

1. Cut the sample into small pieces (0.5-1 cm) and transfer it to a 1.5 mL centrifuge tube.

   Tip: For hair, cut from base of hair; for feathers: select the primary feathers. (Large birds, secondary tail or breast feather can be use).

2. Add 200 µL TL Buffer**,** 25 µL Proteinase K Solution and 20 µL 1M DTT. Mix throughly by vortexing. Incubate 30 min at 60°C with occasional mixing.

3. Add 225 µL Buffer MSL to the sample, mix throughly by vortexing.

4. Centrifuge at maximum speed (>14,000 x g) for 5 minutes.

5. Follow the protocol for sperm (Page 6) from Step 5.

Centrifugal Protocol

Note: Please read through previous sections of this manual before using this protocol.

1. Prepare samples by following the standard protocol in previous sections.

2. For all binding, washing and elution steps. Instead to use the magnetic separation device to collect the Mag-Bind particles, centrifuge the tube or plate at 14,000 x g for 1 minute (for tube) or 3000 x g for 3 minutes to collect the magnetic beads.

Troubleshooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Incomplete resuspension of magnetic particle	Resuspend the magnetic particles by vortexing before use.
	Inefficient cell lysis due to inefficient mix of buffer MSL and sample	Make sure the sample is throughly mixed with BufferMSL.
	SPM Buffer were not prepared correctly.	Prepare the SPM Buffer by adding ethanol according to instruction
	Lose of magnetic beads during opetation	careful not remove the magnetic beads during the operation
	Inefficient cell lysis due to decrease of activity of proteinase k	Add more Proteinase K Solution.
No DNA eluted	SPM Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare SPM Wash Buffer Concentrate as instructed on the label.
Problem with downstream application	Insufficient DNA was used	1. Use more stating material 2. Quantify the purified DNA accurately and use sufficient DNA.
	Excess DNA was used for downstream application	Make sure to use correct amount DNA.