Contents

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Introduction

The Mag-Bind® Forensic DNA Isolation KF96 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 by using Kingfisher instrument. This kit can also be used for the preparation of genomic DNA from mouse tail snips, whole blood, buffy coat, serum, and plasma. 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

Mag-Bind® Forensic DNA Isolation KF96 Kit 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 Mag-Bind® Forensic DNA Isolation KF96 Kit except the Proteinase K, can be stored at 22°C-25°C. Once reconstituted in water, Proteinase K must be stored at -20°C. Under these conditions, performance of all components of the kit are guaranteed at least 18 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	M1429-00	M1429-01	M1429-02
Purification	1 x 96	4 x 96	20 x 96
Mag-Bind Particles S	1.1 mL	4.2 mL	21 mL
Buffer MSL	35 mL	140 mL	700 mL
Buffer TL	45 mL	175 mL	875 mL
SPM Buffer	36mL	144 mL	3 x 300 mL
MP Buffer	20 mL	80 mL	400 mL
Elution Buffer	15 mL	60 mL	2 x 150 mL
Proteinase K	60 mg	240 mg	1.2 g
Proteinase Storage Buffer	4 mL	15 mL	65 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

Before Starting

- Reconstitute Proteinase K with Proteinase Storage Buffer to final concentration at 20mg/ml. Store at -20°C. Vortex vial briefly prior to use.

Important	SPM Buffer must be diluted with absolute ethanol as follows **M1429-00** Add 84 mL ethanol / bottle **M1429-01** Add 336ml ethanol/bottle **M1429-02** Add 700 mL ethanol / bottle
	Prepare **FRESH** Buffer MP/Ethanol as follows. This mixture can only be stored at room temperature for two weeks. **M1429-00** Add 30ml absolute ethanol **M1429-01** Add 120ml absolute ethanol **M1429-02** Add 600 ml absolute ethanol

Forensic DNA Protocol

KingFisher 96 Process

Pipetting Instruction for KingFisher 96 and Mag-Bind Forensic DNA protocol

Plate Type	Plate	Content	Reagent Volume
A	1	Sample/ Lysate according to protocols below.	
A	2	MP Buffer	500 µl
A	3	SPM Buffer	600 µl
A	4	SPM Buffer	600 µl
B	5	Elution Buffer	100 µl

* A= KingFisher 96 DW Plate, B=KingFisher 96 KF Plate

- Prepare lysate by following the use instruction based on sample type.
- Add 500 µl MP Buffer to **Plate 2.**
- Add 600 µl SPM Wash Buffer to **Plate 3.**
- Add 600 µl SPM Wash Buffer to **Plate 4**.
- Add 100 µl Elution Buffer to **Plate 5.**

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. We recommend using OB Specimen Paper (OSP-01 and OSP-02) for spotting blood, This kit can also be used for samples collected by using other specimen collection papers such as FTA cards.

1. Cut or punch out the blood spot (or other sample) from the filter paper. (1ul - 10ul of blood can be used for each spot.) Tear or cut filter into small pieces and place into a microfuge tube.
   Note: Use 3-4 punched cycles (3mm diameter) for each DNA isolation.

2. Add 100ul Buffer TL and 10ul Proteinase K and mix by vortexing. Incubate for 30-45 minutes at 55°C with occasional mixing.

3. Add 110ul Buffer MSL and incubate at 55°C for 10 minutes. Vortex every 2 min to mix.

5. Transfer **200 µl** to a Kingfisher DW Plate.
6. Add 160µl of Ethanol(96-100 %)l followed by 10µl of Mag-Bind particles to the KF Deep well plate. This will be Plate 1 in Kingfisher Protocol.
7. Press start on Kingfisher 96 dried blood spots protocol and load plates accordingly.

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.

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

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 (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. Transfer **400 µl** sample to a KF 96 Deep Well Plate.

5. Add **270µl** absolute ethanol to each sample.

6. Add **10µl** of Mag-Bind particles and mix throughly. This will be Plate 1 in Kingfisher Protocol
7. Press start on Kingfisher 96 Sperm protocol and load plates accordingly.

Protocol For Isolation of Genomic DNA From Buccal Swabs:

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 microcentrifuge tube/ plate and add 400 µL TL to the tube. Add 20 µL Proteinase K solution (20mg/ml). Incubate 60 minutes at 56°C.

3. Transfer 290 µl of lysate into a KingFisher Deep Well Plate.
4. Add 300 µL Buffer MSL to the sample. Mix immediately by vortexing for 30 seconds. Add 400 µl ethanol and 10 µl magnetic beads. This will be plate 1 in the KF protocol.

5. Press start on Kingfisher 96 Buccal Swabs protocol and load plates accordingly.

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 Salvia:

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

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

3. Incubate at 65°C for 30 minutes.

4. Transfer the sample to a KF 96 Deep Well Plate.

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. This will be plate 1 in Kingfisher Protocol.

7. Press start on Kingfisher 96 Saliva protocol and load plates accordingly.

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

3. Transfer 290 µl of lysate into a KingFisher Deep Well Plate without removing insoluable debri. It may be necessary to centrifuge at 4,000 x g to pellet debri..

4. Add 300 µL Buffer MSL to the sample. Mix immediately by vortexing for 30 seconds. Add 400 µl ethanol and 10 µl magnetic beads. This will be plate 1 in the KF protocol.

5. Press start on Kingfisher 96 Buccal Swabs protocol and load plates accordingly

Determination of Yield and Quality

The total DNA yield can be determined by a spectrophotometer using deionized water, Tris-HCl buffer, or Elution Buffer as blank. DNA concentration is calculated as:

[DNA] = (Absorbance$_{260}$) x (0.05 µg/ µL) x (Dilution factor)

The quality of DNA can be assessed by measuring absorbance at both 260 nm and at 280 nm. A ratio of (A_{260}/A_{280}) of 1.7-1.9 corresponds to 85%-95% purity.

Expected yields vary with both amount, and type of tissue used. 30 mg of fresh tissue will yield 10-40 µg DNA with two elutions (each 200 µL).

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.