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Introduction

E.-Z 96® Mag-Bind Blood DNA KF 96 Kit allows rapid and reliable isolation of high-quality genomic DNA from 1-250 µl blood samples. The system combines the reversible nucleic acid-binding properties of Mag-Bind® magnetic particles with the time-proven efficiency of OBI's blood DNA isolation system to provide a fast and convenient blood DNA isolation method. The magnetic particles technology provides high quality DNA that is suitable for direct use in most downstream applications, such as amplification and enzymatic reactions.

Principle

E.Z.N.A.® Mag-Bind 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 whole Blood and cultured cells. 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 Blood DNA Isolation 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 MSL. In case of such an event, heat the bottle at 37°C to dissolve the precipitate.

Kit Contents

Product	M6321-00	M6321-01	M6321-02
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
SPM Buffer	36mL	144 mL	3 x 300 mL
MP Buffer	20 mL	80 mL	400 mL
Elution Buffer	15 mL	60 mL	mL
Proteinase K	60 mg	240 mg	1.2 g
Proteinase Storage Buffer	5 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 Blood 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 **M6321-00** Add 84 mL ethanol / bottle **M6321-01** Add 336ml ethanol/bottle **M6321-02** Add 700 mL ethanol / bottle
	Prepare **FRESH** Buffer MP/Ethanol as follows. This mixture can only be stored at room temperature for two weeks. **M6321-00** Add 30ml absolute ethanol **M6321-01** Add 120ml absolute ethanol **M6321-02** Add 600 ml absolute ethanol

E-Z 96® Mag-Bind® Blood DNA Protocol (1-200µl Blood) for Kingfisher Flex 96 or Kingfisher 96

The procedure below has been optimized for use with FRESH or FROZEN blood samples 1 to 200 µl in volume. Anticoagulated blood or buffy coat can also be used.

Equipment User Provided

- **4 Kingfisher Deep Well Plates**
- **1 Kingfisher 96 Plate**
- **96-100% Ethanol**
- **Rnase A(Optional)**
- **Kingfisher Flex 96 or Kingfisher 96**
- **Kingfisher Tip Comb**
- **Multi Channel Pipettor**
- **Sealing Film**

KingFisher 96 Process

Pipetting Instruction for KingFisher 96 or Kingfisher Flex and Mag-Bind Blood DNA protocol

Table 1

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.**

Sample Preparation

1. **Add blood sample to a Kingfisher deep well plate. Bring the volume up to 200 µl with 10 mM Tris-HCl, PBS, or Elution Buffer provided with this kit.**

2. **Add 20 µl Proteinase K and 5 µl Rnase A (not Provided) to the sample.** Mix throughly by pipetting up and down for 10 times.

3. Add 220 µl Buffer MSL to the Samples and mix by pipetting up and down 10 times.

4. Incubate at 56°C for 20 minutes.

5.
6. Press start on Kingfisher 96 whole blood protocol and load plates accordingly.

7. During Pause Step: Add 300 µl of Ethanol (96-100 %) followed by 10µl of Mag-Bind particles to the KF Deep well plate.

6. Remove Plate containing genomic DNA and store at -20°C

E-Z 96® Mag-Bind® Blood DNA Protocol (1-200µl Blood) for Kingfisher mL

The procedure below has been optimized for use with FRESH or FROZEN blood samples 1 to 200 µl in volume. Anticoagulated blood or buffy coat can also be used.

Table 2

Strip	Content	Reagent Volume
A	Sample/ Lysate according to protocols below.	
B	MP Buffer	500 µl
C	SPM Buffer	600 µl
D	SPM Buffer	600 µl
E	Elution Buffer	100 µl

This protocol can be used for fresh or frozen semen samples with equal efficiency. Frozen samples must to be thawed thoroughly before use.

Sample Preparation

1. **Add blood sample to a 1.5 mL tube . Bring the volume up to 200 µl with 10 mM Tris-HCl, PBS, or Elution Buffer provided with this kit.**

2. **Add 20 µl Proteinase K and 5 µl Rnase A (not Provided) to the sample.** Mix throughly by pipetting up and down for 10 times.

3. Add 20 µl Buffer MSL to the Samples and mix by pipetting up and down 10 times.

4. Incubate at 56°C for 20 minutes.

5. Add 300 µL Ethanol(96-100%) and 10 µL of Mag Bind particles S.

6. Transfer the Sample in Tube Strip A.

7. Press start on Kingfisher mL whole blood protocol and load Strips accordingly to Table 2

8. Remove Plate containing genomic DNA and store at -20°C

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.