Contents

Revised August 2006

Introduction

E.Z.N.A.™ Mag-Bind Blood DNA Kit allows rapid and reliable isolation of high-quality genomic DNA from 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.

Overview

If using the E.Z.N.A.™ Mag-Bind Blood DNA Kit for the first time, please read this booklet in its entirety to become familiar with the procedures. Blood cells are disrupted and then lysed in a specially formulated buffer. DNA is isolated from lysates in one step through its binding to Mag-Bind® particles' surfaces. The magnetic particles are separated from lysates by using a magnet separation device. After two rapid wash steps remove trace contaminants, DNA is eluted in Elution Buffer.

Storage and Stability

Most components of the E.Z.N.A.™ Mag-Bind Blood DNA Kit are stable for at least 24 months from date of purchase when stored at 22°C-25°C. Magnetic Particles Solution C should be stored longterm at 4° C.

Kit Contents

Product Number	M6221-00	M6221-01	M6221-02
Purification Times	5 Preps	50 Preps	200 Preps
Mag-Bind Particles Solution C	55 µl	550 µl	2.2 mL
Buffer MTL	3 mL	30 mL	120 mL
Buffer MSL	3 mL	30 mL	120 mL
MP Buffer	3 mL	20 mL	60 mL
SPM Wash Buffer Concentrate	3 mL	30 mL	75 mL
RNase A	30 µl	280 µl	1.20 mL
Proteinase K	3 mg	30 mg	100 mg
Elution Buffer	1 mL	20 mL	80 mL
Instruction Booklet	1	1	1

Materials to be provided by user

- Nuclease-free 1.5 mL centrifuge tube (for single tube preparation)
- 500 µL Nuclease-free microplate
- 1.2mL deep well plate (EV1772)
- 8-strip caps for 1.2mL deep well plate (EV1774)
- Water bath, incubator or heating block preset at 65° C
- Absolute ethanol (96%-100%)
- Magnetic separation device for microcentrifuge tubes or microplates

Before Starting

- Please read this bookly thoroughly to become familiar with the Mag-Bind® Blood DNA Kit procedures.
- Dilute Proteinase K with Elution Buffer as follows and **store at -20°C**.

M6221-00	Add 75 µl Elution Buffer
M6221-01	Dissolve with 625 µl Elution Buffer
M6221-02	Dissolve with 2.5 mL Elution Buffer

- Dilute SPM Wash Buffer with absolute ethanol (96%-100%) as follows and **store at room temperature**:

M6221-00	Add 7 mL absolute ethanol (96%-100%)
M6221-01	Add 42 mL absolute ethanol (96%-100%)
M6221-02	Add 175 mL absolute ethanol (96%-100%)

- **Prepare FRESH Buffer MP/Ethanol as follows. This mixture can only be stored at room temperature for two weeks**:

M6221-00	Add 3 mL absolute ethanol (96%-100%)
M6221-01	Add 20 mL absolute ethanol (96%-100%)
M6221-02	Add 60 mL absolute ethanol (96%-100%)

- Shake or vortex the Mag-Bind® solution to fully resuspend the particles. The particles must be fully suspended during use to assure proper binding.

E.Z.N.A. Mag-Bind Blood DNA Protocol (1-100 µl Blood)

The procedure below has been optimized for use with FRESH or FROZEN blood samples 1 to 100 µl in volume. Anticoagulated blood or buffy coat can also be used.

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

2. **Add 210 µl Buffer MSL, 10 µl Proteinase K and 5 µl RNase A solution.** Mix throughly by vortexing or pipetting up and down 20 times.

3. **Incubate sample at 65° C for 20 min**. Briefly vortex the tube during incubation.

4. Cool the sample to room temperature by incubating at room temperature for 5 minutes.

5. **Add 300 µl absolute ethanol to the lysate**. Mix gently by pipetting up and down 20 times or vortexing.

6. **Add 10 µl Mag-Bind Particles Solution C to the sample**. Mix gently by rotating, vortexing or pipetting up and down 20 times.

7. **Incubate at room temperature for 10 minutes.**

8. Place the tube on a magnetic separation device to magnetize the magnetic particles. Incubate at room temperature for 10 minutes.

9. **Completely remove and discard the cleared supernatant. Remove any droplets of liquid from the wall of the tube.**

10. Remove the tube containing the Mag-Bind particles from the magnetic separation device. **Add 500 µl Buffer MP/Ethanol mixture to each sample.**

    Note: MP/Ethanol mix has to be prepared freshly.

11. **Resuspend the Mag-Bind particles pellet by vortexing or pipetting up and down.** Incubate 5 minutes at room temperature. During incubation, mix the sample several times by vortexing or pipetting up and down.

    Complete resuspension of the Mag-Bind particles pellet by pipetting up and down or vortexing is critical to obtain good results.

12. Place the tune onto the magnetic separation device to magnetize the Mag-Bind particles.

13. Completely remove and discard the cleared supernatant.

14. Remove the tube containing the Mag-Bind particles from the magnetic separation device. Add 1000 µl SPM Buffer to each sample.

15. Resuspend the Mag-Bind particles pellet by vortexing. Incubate 3 minutes at room temperature. Complete resuspension of the Mag-Bind particles pellet by pipetting up and down or vortexing is critical to obtain good results.

16. Place the tube onto the magnetic separation device to magnetize the magnetic particles.

17. Completely remove and discard the cleared supernatant.

18. **Optional:** Add 400 µl absolute ethanol and resuspend the Mag-Bind particles by vortexing or pipetting up and down. Magnetize the Mag-Bind particles then remove the supernatant.

19. Leave the tube to air dry on the magnetic separation device for 5-10 minutes. Remove any residue liquid with a pipettor.

20. Remove the tube from the magnetic separation device. **Add 50-200 ul Elution Buffer or water to elute DNA from the Mag-Bind particles.** Incubate 5 minutes at 70°C.

21. Resuspend the Mag-Bind particles by vortexing for 3 minutes (tubes) or pipetting up an down 50 times (microplate) .

22. Place the tube onto a magnetic separation device to magnetize the Mag-Bind particles.

23. Transfer the cleared supernatant containing purified DNA to a new 1.5 mL tube.

Mag-Bind Blood DNA 96-well Protocol (1-100µl Blood)

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.

1. **Add blood sample to a 1.2 mL deep well plate. Bring the volume up to 200 µl with 10 mM Tris-HCl, PBS, or Elution Buffer provided with this kit.**

2. **Prepare the master mix as follow: 10 µl Proteinase K , 200 µl Buffer MSL,5 µl RNase A and 10 µl Mag-Bind Particles Solution**. Add 220 µl the master mix to a well of samples. Seal the plate with caps. Mix throughly by vortexing.

3. **Incubate sample at 65° C for 20 min.** Briefly vortex the few times during incubation or use a rocker shaker.

4. Cool the sample to room temperature by sit the plate at room temperature for 5 minutes.

5. **Add 300 µl absolute ethanol to the lysate. Seal the plate with new caps (supplied). Mix the sample by shaking or vortex the plate gently (side to side) for 1 minute.**

6. **Incubate at room temperature for 10 minutes.**

7. Transfer 360 µl of the sample into a 96-well microplate. Place the plate on a magnetic separation device to magnetize the magnetic particles. Incubate at room temperature for 10 minutes.

8. Remove and discard the cleared supernatant. Transfer the remaining of sample (360 µl) into the microplate.

9. Place the plate on a magnetic separation device to magnetize the magnetic particles. Incubate at room temperature for 10 minutes. **Remove any droplets of liquid from the wall of the tube with the pipettor.**

10. Remove the plate containing the Mag-Bind particles from the magnetic separation device. **Add 400 µl Buffer MP/Ethanol Mixture to each sample.**

    Note: MP/Ethanol mix has to be prepared freshly.

11. **Resuspend the Mag-Bind particles pellet by pipetting up and down 20 times. Incubate 5 minutes at room temperature.**

    During incubation, mix the sample several times by pipetting up and down. Complete resuspension of the Mag-Bind particles pellet by pipetting up and down is critical to obtain good results.

12. **Place the plate onto the magnetic separation device to magnetize the Mag-Bind particles.**

13. **Completely remove and discard the cleared supernatant.**

14. Remove the plate containing the Mag-Bind particles from the magnetic separation device. Add 400 µl SPM Wash Buffer to each sample.

15. Resuspend the Mag-Bind particles pellet by pipetting up and down 20 times. Incubate 1 minutes at room temperature.

    Complete resuspension of the Mag-Bind particles pellet by pipetting up and down or vortexing is critical to obtain good results.

16. Place the plate onto the magnetic separation device to magnetize the magnetic particles.

17. Completely remove and discard the cleared supernatant.

18. Remove the plate containing the Mag-Bind particles from the magnetic separation device. Add another 400 µl SPM Wash Buffer to each sample.

19. Resuspend the Mag-Bind particles pellet by vortexing.

20. Place the plate onto the magnetic separation device to magnetize the magnetic particles.

21. Completely remove and discard the cleared supernatant.

22. **Optional:** Add 400 µl absolute ethanol and resuspend the Mag-Bind particles by pipetting up and down 20 times. Magnetize the Mag-Bind particles then remove the supernatant.

23. Leave the plate to air dry on the magnetic separation device for 5-10 minutes. Remove any residue liquid with a pipettor.

24. Remove the plate from the magnetic separation device. Add 50-200 ul Elution Buffer or water to elute DNA from the Mag-Bind particles. Seal the plate and incubate 5 minutes at 65°C.

25. Resuspend the Mag-Bind particles by vortexing for 3 minutes (tubes) or pipetting up an down 50 times (microplate) .

26. Place the tube onto a magnetic separation device to magnetize the Mag-Bind particles.

27. Transfer the cleared supernatant containing purified DNA to a new 1.5 mL tube.

Mag-Bind Blood DNA Protocol (100-200 µl Blood)

The procedure below has been optimized for use with FRESH or FROZEN blood samples 100 to 200 µl in volume. Anticoagulated blood or buffy coat can also be used.

1. Add blood sample to a nuclease-free microcentrifuge tube. **Bring the volume up to 300 µl with 10 mM Tris-HCl, PBS, or Elution Buffer provided with this kit.**

2. **Add 320 µl Buffer MSL, 10 µl Proteinase K and 5 µl RNase A solution.** Mix throughly by vortexing or pipetting up and down 20 times.

3. **Incubate sample at 65° C for 20 min**. Briefly vortex the tube during incubation.

4. Cool the sample to room temperature by incubating at room temperature for 5 minutes.

5. **Add 430µl absolute ethanol to the lysate**. Mix gently by pipetting up and down 20 times or vortexing.

6. **Add 10 µl Mag-Bind Particles Solution C to the sample**. Mix gently by rotating, vortexing or pipetting up and down 20 times.

7. **Incubate at room temperature for 10 minutes.**

8. Place the tube on a magnetic separation device to magnetize the magnetic particles. Incubate at room temperature for 10 minutes.

9. **Completely remove and discard the cleared supernatant. Remove any droplets of liquid from the wall of the tube.**

10. Remove the tube containing the Mag-Bind particles from the magnetic separation device. **Add 500 µl Buffer MP/Ethanol mixture to each sample.**

    Note: MP/Ethanol mix has to be prepared freshly.

11. **Resuspend the Mag-Bind particles pellet by vortexing or pipetting up and down.** Incubate 5 minutes at room temperature. During incubation, mix the sample several times by vortexing or pipetting up and down.

    Complete resuspension of the Mag-Bind particles pellet by pipetting up and down or vortexing is critical to obtain good results.

12. Place the tune onto the magnetic separation device to magnetize the Mag-Bind particles.

13. Completely remove and discard the cleared supernatant.

14. Remove the tube containing the Mag-Bind particles from the magnetic separation device. Add 1000 µl SPM Buffer to each sample.

15. Resuspend the Mag-Bind particles pellet by vortexing. Incubate 3 minutes at room temperature. Complete resuspension of the Mag-Bind particles pellet by pipetting up and down or vortexing is critical to obtain good results.

16. Place the tube onto the magnetic separation device to magnetize the magnetic particles.

17. Completely remove and discard the cleared supernatant.

18. **Optional:** Add 400 µl absolute ethanol and resuspend the Mag-Bind particles by vortexing or pipetting up and down. Magnetize the Mag-Bind particles then remove the supernatant.

19. Leave the tube to air dry on the magnetic separation device for 5-10 minutes. Remove any residue liquid with a pipettor.

20. Remove the tube from the magnetic separation device. **Add 50-200 ul Elution Buffer or water to elute DNA from the Mag-Bind particles.** Incubate 5 minutes at 70°C.

21. Resuspend the Mag-Bind particles by vortexing for 3 minutes (tubes) or pipetting up an down 50 times (microplate) .

22. Place the tube onto a magnetic separation device to magnetize the Mag-Bind particles.

23. Transfer the cleared supernatant containing purified DNA to a new 1.5 mL tube.

Protocol for Dried Blood, Body Fluids and Sperm Spot

Dried **blood, body fluids, and sperm** samples on filter paper can be processed using the following method. We recommend using OBI Specimen Paper (OBP-01 and OBP-02) for spotting blood, as this unique filter paper disintegrates when incubated in aqueous buffers, allowing for the efficient recovery of DNA. This kit can also be used for samples collected using other specimen collection papers. Please note that this protocol will need TL Buffer (not supplied with this kit). TL Buffer can be purchased separately from OBI or its distributors.

Before starting

Bring frozen samples and OB Protease solution to room temperature, preheat an aliquot of Elution Buffer (approximately 0.5 mL per sample) at 65° C.

1. Cut or punch out the blood (or other sample) spot from the filter paper. Tear or cut filter into small pieces and place into a 1.5 or 2.0 mL centrifuge tube (not provided).

   Note: Use 1-4 punched circles (3 mm diameter) for each DNA isolation.

2. **Add 200 µl Buffer TL to 1-4 punched filter paper circles (3 mm)**. Follow by addition of 10 µl Proteinase K solution. Incubate mixture at 55°C for 45-60 minutes. Mix the samples several times during incubation by vortexing.

3. Briefly centrifuge the centrifuge tube to bring down any liquid droplets from inside the lid.

4. **Add 220 µl Buffer MSL, close the lid and mix throughly by vorexting 20 sec at maximum speed.**

5. **Place the tube in a heating block or waterbath preset at 65°C**. **Incubate for 15 minutes.** Vortex the tube for 10 seconds several times during incubation.

6. Briefly centrifuge the centrifuge tube to bring down any liquid droplets from inside of the lid. Cool the sample to room temperature by incubation at room temperature for 5 minutes.

   Note: For maximum yield, collect any remaining liquid from paper and transfer entire sample, including paper, to a Homogenizer Column (not supplied) and centrifuge at 20,000 x g for 2 minutes to collect all the lysates. Homogenizer Columns (Product No. HCR-001 an HCR-003) can be purchased separately from Omega Bio-Tek.

7. **Transfer the cleared sample into a new 1.5 mL tube or 96-well microplate**.

8. Add 0.7 volume of absolute ethanol to lysate. Mix gently by pipetting up and down 20 times or vortexing.

9. Add 10 µl Mag-Bind Particle Solution C to the sample. Mix gently by vortexing or pipetting up and down 20 times. Incubate at room temperature for 10 minutes

10. Place the tube or microplate on a magnetic separation device to magnetize the magnetic particles. Lysate will clear when the Mag-Bind particles have completely moved toward the magnet.

11. Completely remove and discard the cleared supernatant. Remove any droplets of liquid from the wall of the tube or well with the pipettor.

12. Continue the wash and elute step by following step 10-24 in Mag-Bind Blood Protocol (page 5-6).

Trouble Shooting Guide

Problem	Cause	Suggestions
Low DNA yield	Incomplete resuspension of magnetic particle	Resuspend the magnetic particles by vortexing before use.
	Frozen blood samples not mixed properly after thawing.	Thaw the frozen blood at room temperature and gently mix the blood by inverting.
	Loss the Mag-Bind® particles during operation	Carefully avoid remove the Mag-Bind® particles during aspiration
	DNA remains bound to Mag-Bind® Particles	Increase elution volume and incubate at 65°C for 5 min elution. Pipet up and down for 50-100 times.
	DNA washed off.	Dilute MGC 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.	SPM Buffer must be at room temperature.
	Ethanol carry-over	Dry the Mag-Bind® particle before elution.