Contents

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Introduction

E.Z.N.A.™ Mag-Bind Blood DNA Kit allows rapid and reliable isolation of high-quality genomic DNA from 2mL-10ml 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 speciafMaxlly 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 long term at 4° C.

Kit Contents

Product Number	M6213-00	M6213-01	M6213-02
Purification	10 mL	50 mL	250 mL
Mag-Bind Particles Solution C	660 µL	3.2 ml	16 ml
Buffer MSL	20ml	100 ml	500 ml
MP Buffer	10ml	80 ml	400 ml
SPM Wash Buffer	9 ml	75 ml	3 x 300ml
RNase A	130 µl	350 µl	1.75 ml
Proteinase K	12 mg	60 mg	300 mg
Proteinase Storage Buffer	625 µl	6 ml	20 ml
Elution Buffer	7 ml	50 ml	150 ml
Instruction Booklet	1	1	1

Before Starting

Please read this bookly thoroughly to become familiar with the Mag-Bind® Blood DNA Kit procedures.

- Dilute **Proteinase K** with **Proteinase Storage Buffer** as follows and **store at -20°C**.

M6213-00	Add 600 µl Proteinase Storage Buffer
M6213-01	Dissolve with 3 ml Proteinase Storage Buffer
M6213-02	Dissolve with 15 ml Proteinase Storage Buffer

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

M6213-00	Add 21 ml absolute ethanol (96%-100%)
M6213-01	Add 175 ml absolute ethanol (96%-100%)
M6213-02	Add 700 ml absolute ethanol (96%-100%)

- Prepare **Buffer MP/Ethanol** as follows, this mixture has to be freshly prepared and can be **stored at room temperature for 2 weeks**

M6213-00	Add 15 ml absolute ethanol (96%-100%)
M6213-01	Add 120ml absolute ethanol (96%-100%)
M6213-02	Add 600 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.

Mag-Bind Blood DNA Maximum Yield Protocol (2ml-6ml)

Materials to be provided by user

- Nuclease-free 50 ml centrifuge tube
- Water bath, incubator or heating block preset at 65° C
- Absolute ethanol (96%-100%)
- Magnetic separation device for 50 ml tube

The procedure below has been optimized to yield maximum DNA from FRESH or FROZEN blood samples 2 to 6 ml in volume. Yield an quality of DNA depend on the storage of the blood. Fresh blood normally give better result. For short term storage, collect the blood in tubes contains EDTA as anticoagulant and store at 2-8°C for up to 7 days. For long-term storage, collect the blood in tubes contains anticoagulant and store at -70°C.

Volume of Buffers and Reagents to Add to Various Volume of Whole Blood Sample

Sample Volume	MSL Buffer	PBS	Proteinase K	RNase A	Ethanol
2 ml	3.3 ml	1.3 ml	50µl	20 µl	4.45 ml
3 ml	5 ml	2 ml	75µl	20 µl	6.75 ml
4 ml	6.6 ml	2.6 ml	100µl	30 µl	10.4 ml
5 ml	8.4 ml	3.4 ml	150µl	30 µl	11.34 ml
6 ml	9.9 ml	3.9 ml	200µl	40 µl	13.35 ml

1. Place a empty, uncapped 50 ml conical tube into a 50ml tube holder..

2. Add 1-6 ml blood sample to 50ml tube.

3. Add indicated volume of PBS, MSL, Proteinase K and RnaseA showed in the table to the sample. Mix throughly by vortexing

4. Incubate sample at 65° C for 20 min. Briefly vortex the tube few times during incubation. Cool the sample to room temperature by incubating at room temperature for 5 minutes.

5. Add indicated volume of absolute ethanol showed in the table to the lysate. Mix throughly by inverting or voretxing.

6. Add 300 µl Mag-Bind Particles Solution C to the sample. Mix gently by inverting, shaking or pipetting up and down 20 times. Incubate at room temperature for 10 minutes.

   Note: Complete resuspension of cell pellet is vital for obtaining good DNA yields.

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

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

9. Remove the tube containing the Mag-Bind particles from the magnetic separation device. Add 5 ml Buffer MP/Ethanol Mixture to the sample.

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

10. Resuspend the Mag-Bind particles pellet by vortexing. Incubate 3 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.*

11. Transfer the sample to a new 50 ml tube and Incubate the sample at room temperature for 5 minutes.

12. Place the tune onto the magnetic separation device to magnetize the Mag-Bind particles.Completely remove and discard the cleared supernatant.

13. Remove the tube containing the Mag-Bind particles from the magnetic separation device. Add 10ml SPM Buffer to the sample.

14. Resuspend the Mag-Bind particles pellet by vortexing. Incubate 3 minutes at room temperature. *Complete resuspension of the Mag-Bind particles pellet is critical to obtain good results.*

15. Place the tube onto the magnetic separation device to magnetize the magnetic particles. Completely remove and discard the cleared supernatant.

16. Optional: Wash magnetic particles with SPM by repeating step 13-15.

17. Leave the tube to air dry on the magnetic separation device for 5 minutes. Remove any residue liquid with a pipettor. **Do not over dry the pellet**.
18. Remove the tube from the magnetic separation device. Add 2.5 ml Elution Buffer or water to elute DNA from the Mag-Bind particles. Mix throughly by vortexing for 30 seconds. Incubate 10 minutes at 65°C.

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

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

Mag-Bind Blood DNA Maximum Yield Protocol (7ml-10ml)

Materials to be provided by user

- Nuclease-free 50 ml centrifuge tube
- Water bath, incubator or heating block preset at 65° C
- Absolute ethanol (96%-100%)
- Magnetic separation device for 50 ml tubes

The procedure below has been optimized to yield maximum DNA from FRESH or FROZEN blood samples 7 to 10 ml in volume. Yield an quality of DNA depend on the storage of the blood. Fresh blood normally give better result. For short term storage, collect the blood in tubes contains EDTA as anticoagulant and store at 2-8°C for up to 7 days. For long-term storage, collect the blood in tubes contains anticoagulant and store at -70°C.

Volume of Buffers and Reagents to Add to Various Volume of Whole Blood Sample

Sample Volume	MSL Buffer	PBS	Proteinase K	RNase A	Ethanol
7 ml (3.5ml x 2)	5.8 ml x 2	2.3 ml x 2	87.5µl x 2	25µl x 2	7.83 ml x 2
8 ml (4ml x 2)	6.6 ml x 2	2.6ml x 2	100µl x 2	25µl x 2	10.4 ml x 2
9 ml (4.5ml x 2)	12ml x 2	3 ml x 2	112µl x 2	30µl x 2	13.2 ml x 2
10ml (5 ml x 2)	8.4 ml x 2	3.4 ml x 2	125µl x 2	30µl x 2	11.3 ml x 2

1. Place two empty, uncapped 50 ml conical tubes into a 50ml tube holder.

2. Equally divide the blood sample and transfer the sample into twp 50 ml tubes. For example: For 7ml blood, transfer 3.5ml blood into each tube.

3. Add indicated volume of PBS, MSL, Proteinase K and RnaseA showed in the table to the sample. Mix throughly by vortexing.

4. Incubate sample at 65° C for 20 min. Briefly vortex the tube few times during incubation. Cool the sample to room temperature by incubating at room temperature for 5 minutes.

5. Add indicated volume of absolute ethanol showed in the table to the lysate. Mix throughly by inverting or voretxing.

6. Add 300 µl Mag-Bind Particles Solution C to the sample. Mix gently by inverting, shaking or pipetting up and down 20 times. Incubate at room temperature for 10 minutes.

   Note: Complete resuspension of cell pellet is vital for obtaining good DNA yields.

7. Place the tubes on a magnetic separation device to magnetize the magnetic particles. Lysate will clear when the Mag-Bind particles have completely moved toward the magnet. Completely remove and discard the cleared supernatant.

8. Remove the tubes containing the Mag-Bind particles from the magnetic separation device. Add 5 ml Buffer MP/Ethanol Mixture to the sample.

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

9. Resuspend the Mag-Bind particles pellet by vortexing. Incubate 3 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.*

10. **Transfer and combine** suspended Mag-Bind Particles into a new 50 ml tube. **Incubate the sample at room temperature for 5 minutes.**

11. Place the tube onto the magnetic separation device to magnetize the Mag-Bind particles. Completely remove and discard the cleared supernatant.

12. Remove the tube containing the Mag-Bind particles from the magnetic separation device. Add 10 ml SPM Buffer to each sample.

13. Resuspend the Mag-Bind particles pellet by vortexing. Incubate 3 minutes at room temperature. *Complete resuspension of the Mag-Bind particles pellet is critical to obtain good results.*

14. Place the tube onto the magnetic separation device to magnetize the magnetic particles. Completely remove and discard the cleared supernatant

15. Optional: Wash magnetic particles with SPM by repeating step 12-14.

16. Leave the tube to air dry on the magnetic separation device for 5 minutes. Remove any residue liquid with a pipettor. **Do not over dry the pellet**.

17. Remove the tube from the magnetic separation device. Add 2.5 ml Elution Buffer or water to elute DNA from the Mag-Bind particles. Incubate 10 minutes at 65°C. Mix throughly by vortexing for 30 seconds. Incubate 10 minutes at 65°C.

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

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

Troubleshooting

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 SPM Wash Buffer by adding appropriate volume of absolute ethanol prior to use (page 3).
	MP/ethanol mix is old	Prepare MP/Ethanol mix freshly
Problems in downstream applications	Salt carry-over.	SPM Buffer must be stored at room temperature.
	Ethanol carry-over	Dry the Mag-Bind® particle before elution.