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Introduction

E.-Z 96® Mag-Bind Blood Midi DNA Kit allows rapid and reliable isolation of high-quality genomic DNA from 300-500 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.

Overview

If using the E.-Z 96® Mag-Bind Blood DNA Midi 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 few rapid wash steps remove trace contaminants, DNA is eluted in Elution Buffer.

Storage and Stability

All components of the E.-Z 96® Mag-Bind Blood Midi DNA Kit, except Magnetic particles, are stable for at least 12 months from date of purchase when stored at 22°C-25°C. Magnetic Particles Solution C should be stored long term at 4° C. Once reconstituted in water, Proteinase K must be stored at -20°C. For long term storage, store RNase A at -20°C.

Kit Contents

Product Number	M6212-00	M6212-01	M6212-02
Purification	1 x 96	4 x 96	20 x 96
Mag-Bind Particles Solution F	2.1 mL	8.4 mL	42 mL
Buffer MSL	60 mL	240 mL	2 x 600 mL
MP Buffer*	20 mL	100 mL	500 mL
SPM Wash Buffer Concentrate	30 mL	160 mL	2 x 300 mL
Proteinase K	50 mg	200 mg	1g
Proteinase Storage Buffer	3 ml	12 ml	55 ml
Elution Buffer	30 mL	120 mL	600 mL
Instruction Booklet	1	1	1

*Prepare fresh MP Buffer by adding ethanol prior the use. See instruction on page 4.

Materials to be Provided By User

- Water bath, incubator or heating block preset at 65° C
- Absolute ethanol (96%-100%)
- Magnetic separation device for 96 deep well plate (OBI Product # MSD-01B)
- 2.2 ml deep-well plate (Cat# EZ9604)
- 1.2 ml Round-well plate (Process plate for MSD-01B)
- Sealing film (AC1200)

Before Starting

Please read this bookly thoroughly to become familiar with the E.-Z 96® Mag-Bind Blood DNA Kit procedures.

Important	Dilute **Proteinase K** with Proteinase Storage Buffer as follows and store at -20°C. **M6212-00** Dissolve with 2.5 ml Proteinase Storage Buffer **M6212-00** Dissolve with 10 ml Proteinase Storage Buffer **M6212-00** Dissolve with 50 ml Proteinase Storage Buffer
	Dilute **SPM Wash Buffer** Concentrate with **absolute ethanol** (96%-100%) as follows and store at room temperature **M6212-00** Add 70 mL absolute ethanol (96%-100%) **M6212-01** Add 240 mL absolute ethanol (96%-100%) **M6212-02** Add 700 mL absolute ethanol (96%-100%)
	Prepare FRESH Buffer MP/Ethanol: For each set of 96 samples, mix 16 ml Buffer MP with 24 ml absolute ethanol (96-100%). This mixture can only be stored at room temperature for two weeks:
	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 96® Mag-Bind Blood Midi DNA Protocol

The procedure below has been optimized for use with FRESH or FROZEN whole blood or bone marrow using up to 500 µl in volume. Anticoagulated blood or buffy coat can also be used.

Sample Preparation

1. **Add blood sample to a 2.2 mL deep well plate. Bring the volume up to 500 µl with 10 mM Tris-HCl, PBS, or Elution Buffer provided with this kit.**

2. **Prepare the master mix as follow (for 96 samples): 2.5 mL Proteinase K , 50 mL Buffer MSL, and 2.0 mL Mag-Bind® Particles Solution**. Add 525 µl of this master mix to each well of samples. Mix throughly by pipetting up and down for 20 times.

3. **Incubate sample at 65°C for at least 10 min.** Briefly mix the sample once by pipetting up and down 5 times during incubation.

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

5. **Add 700 µl absolute ethanol to the lysate.** Mix the sample by pipetting up and down for 20 times.

6. **Incubate at room temperature for 3 minutes.** Briefly mix the sample once by pipetting up and down 5 times during incubation.

DNA Binding

7. **Transfer 680 µl of the sample into a 96-well process plate.** Place the plate on a magnetic separation device to magnetize the magnetic particles. Sit for 10-15 minutes or until magnetic particles are fully magnetized. Aspirate and discard the supernatant

   Note: The time to fully magnetize the beads can be variable depend on the type of magnet and 96-well process plate. Run a test prior the experiment to determine the time for beads settling. If MSD-01B is used, the magnetic particles should be magnetized to the sidewall of each well adjacent to the magnet. The volume of the sample can be processed each transfer can also be adjusted based the magnet configuration and 96-well process plate.

8. Transfer the another 680µl of lysate from step 5 into the 96-well process plate. . Place the plate on a magnetic separation device to magnetize the magnetic particles. Sit for 10-15 minutes or until magnetic particles are fully magnetized. Aspirate and discard the supernatant. Repeat step 8 until all the lysate are been transferred.

9. **Remove and discard the supernatant.** Remove any droplets of liquid drop from the bottom of the each well with the pipettor.

Wash the Magnetic Particles

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

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

11. Resuspend the magnetic particles pellet by shaking or vortsing. Incubate at room temperature for 2 minutes.

    Note: Completely resuspension of the magnetic particles pellet is critical to obtain high quality of DNA.

12. **Place the plate on a magnetic separation device to magnetize the magnetic particles**. Sit at room temperature for 10-15 minutes or until magnetic particles are fully pelleted.

13. **Remove and discard the cleared supernatant.** Remove any droplets of liquid from bottom of the well with the pipettor.

14. Remove the plate containing the magnetic particles from the magnetic separation device. **Add 400 µl SPM Wash Buffer diluted with ethanol to each sample.**

15. Resuspend the magnetic particles pellet by shaking or vortexing. Incubate for 1 minutes at room temperature.

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

17. Completely remove and discard the cleared supernatant. Do not disturb the Magnetic Particles.

18. Remove the plate containing the magnetic particles from the magnetic separation device. **Add 400 µl SPM Wash Buffer diluted with ethanol to each sample.**

19. Resuspend the magnetic particles pellet by shaking or vortexing.

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

21. Completely remove and discard the cleared supernatant. Do not disturb the Magnetic Particles.

22. Remove the cleared supernatant by pipetting.

23. **Optional: Leave the plate on the magnetic separation device** . **Add 400 µl absolute ethanol and incubate at room temperature for 1 minute.** It is not

necessary to resuspend the magnetic particles. Aspirate the liquid.

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

    Note: Heating is permitted to dry the magnetic particles faster.

DNA Elution

25. Remove the plate from the magnetic separation device. **Add 200-300 ul Elution Buffer to elute DNA from the magnetic particles.** Resuspend the magnetic particles by pipetting up and down 50 times.

26. Incubate 10-20 minutes at 65°C.

27. Place the tube onto a magnetic separation device to magnetize the Magnetic particles.

28. Transfer the cleared supernatant containing purified DNA to a new microplate (not supplied). Store the DNA at -20°C.

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