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Introduction

E.-Z 96® Mag-Bind Blood DNA 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.

Overview

If using the E.-Z 96® 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 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 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	M6211-00	M6211-01	M6211-02
Purification	1 x 96	4 x 96	20 x 96
Mag-Bind Particles Solution C	1.1 mL	4.2 mL	21 mL
Buffer MSL	33 mL	125 mL	800 mL
MP Buffer	20 mL	80 mL	400 mL
SPM Wash Buffer Concentrate	30 mL	120 mL	2 x 300 mL
RNase A	500 µl	2 ml	10 ml
Proteinase K	25 mg	100 mg	500 mg
Proteinase Storage Buffer	3 mL	6 mL	30 mL
Elution Buffer	30 mL	120 mL	600 mL
Instruction Booklet	1	1	1

Materials to be Provided By User

- Water bath, incubator or heating block preset at 65° C
- Absolute ethanol (96%-100%)
- Magnetic separation device for microplates (OBI Product # MSD-01)
- 500 µl 96-well Round-bottom plate (Cat# EZ9604)
- 1.2 ml deep well plate (Cat# SSI1780)
- Sealing film (AC1200)

Before Starting

Please read this booklet 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.** **M6211-00** Dissolve with 1ml Proteinase Storage Buffer **M6211-00** Dissolve with 4ml Proteinase Storage Buffer **M6211-00** Dissolve with 20ml Proteinase Storage Buffer
	Dilute SPM Wash Buffer Concentrate with absolute ethanol (96%-100%) as follows and store at room temperature **M6211-00** Add 70 mL absolute ethanol (96%-100%) **M6211-01** Add 280 mL absolute ethanol (96%-100%) **M6211-02** Add 700 mL absolute ethanol (96%-100%)
	Prepare Buffer MP/Ethanol solution as following: **M6211-00** Add 30 mL absolute ethanol (96%-100%) **M6211-01** Add 120 mL absolute ethanol (96%-100%) **M6211-02** 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.

E-Z 96® Mag-Bind® Blood DNA Protocol (1-100µl 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.

Sample Preparation

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. **Add 20 µl Proteinase K and 5 µl Rnase A to the sample.** Mix throughly by pipetting up and down for 20 times.

3. Add 200 µl Buffer MSL to the Samples and mix by pipetting up and down 20 times.

4. **Incubate sample at 65°C for 20 min.** Briefly mix the sample once by pipetting up and down 5 times during incubation.

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

6. **Add 300 µl absolute ethanol and 10 µl magnetic beads to the lysate.** Mix the sample by pipetting up and down for 20 times.

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

DNA Binding

8. **Transfer 360 µl of the sample into a 96 round-bottom 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 pelleted.

   Note: If MSD-01 is used, the magnetic particles should be pelleted at the corner of each well to the magnet. If

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

10. **Place the plate on a magnetic separation device to magnetize the magnetic particles**. Sit at room temperature for 5-10 minutes or until magnetic particles are fully pelleted.

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

Wash the Magnetic Particles

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

13. Resuspend the magnetic particles pellet by pipetting up and down 20 times. Incubate at room temperature for 5 minutes. During incubation, mix the sample one time by pipetting up and down10 times.

    Note: Completely resuspension of the magnetic particles pellet is critical to obtain good results.

14. **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.

15. **Remove and discard the cleared supernatant.** Completely remove any droplets of liquid from the wall of the each well with the pipettor.

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

17. Resuspend the magnetic particles pellet by pipetting up and down 20 times. Incubate for 1 minutes at room temperature.

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

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

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

21. Resuspend the magnetic particles pellet by pipetting up and down 20 times.

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

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

24. Remove the cleared supernatant by pipetting.

25. **Optional: Add 400 µl absolute ethanol and resuspend the magnetic particles by pipetting up and down 20 times.** Magnetize the magnetic particles and then aspirate the supernatant.

26. 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

27. Remove the plate from the magnetic separation device. **Add 100-200 ul Elution Buffer or water to elute DNA from the magnetic particles.** Resuspend the magnetic particles by pipetting up and down 50 times.

28. Incubate 5 minutes at 65°C.

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

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

E-Z 96® Mag-Bind® Blood DNA Protocol (100-250µl Blood)

Sample Preparation

1. **Add blood sample to a 1.2 mL deep well plate. Bring the volume up to 300 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 to the sample. Mix throughly by pipetting up and down for 20 times.**

3. Add 300 µl Buffer MSL to the Samples and mix by pipetting up and down 20 times .

4. **Incubate sample at 65°C for 20 min.** Briefly mix samples one time by pipetting up and down 10 times during incubation or use a rocker shaker.

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

DNA Binding

6. **Add 430 µl absolute ethanol and 10 µl magnetic beads to the lysate to the lysate. Mix throughly by pipetting up and down 20 times.** Sit at room temperature for 5 minutes.

7. Transfer 360 µl of the sample into a 96 well round-bottom plate. Place the plate on a magnetic separation device to magnetize the magnetic particles. Sit for 5-10 minutes or until magnetic particles are fully pelleted.

   Note: If MSD-01 is used, the magnetic particles should be pelleted at the corner of each well to the magnet.

8. **Place the plate on a magnetic separation device to magnetize the magnetic particles. Sit for 5-10 minutes or until magnetic particles are fully pelleted.**

9. **Remove and discard the cleared supernatant.**

10. **Repeat step 6-8 until remaining sample are completely transferred into the Binding Plate.** Completely remove any droplets of liquid from the wall of the each well with the pipettor.

Wash the Magnetic Particles

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

12. **Resuspend the magnetic Particles pellet by pipetting up and down 20 times. Incubate at room temperature for 5 minutes.** During incubation, mix the sample one time by pipetting up and down10 times.

    Note: Completely resuspension of the magnetic particles pellet is critical to obtain good results.

13. **Place the plate on a magnetic separation device to magnetize the magnetic particles**. Sit at room temperature for 5-10 minutes or until magnetic particles are fully pelleted.

14. **Remove and discard the cleared supernatant.** Completely remove any droplets of liquid from the wall of the each well with the pipettor.

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

16. Resuspend the magnetic particle pellet by pipetting up and down 20 times. Incubate for 1 minutes at room temperature.

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

18. Completely remove and discard the cleared supernatant. Do not disturb the magnetic Particles.

19. Remove and discard the cleared supernatant by pipetting.

20. **Optional: Add 400 µl absolute ethanol and resuspend the magnetic particles by pipetting up and down 20 times.** Magnetize the magnetic particles and then aspirate the supernatant.

21. 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

22. Remove the plate from the magnetic separation device. **Add 100-200 ul Elution Buffer or water to elute DNA from the Magnetic® particles.** Resuspend the magnetic particles by pipetting up and down 50 times.

23. Incubate 5 minutes at 65°C.

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

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

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 Proteinase K solution to room temperature.
- Preheat an aliquot of Elution Buffer (approximately 0.5 mL per sample) at 65° C.
- **Prepare the Mag-Bind Particles/Ethanol mixture**: For each 96 set of samples, add 2.8 ml absolute ethanol (96-100%) with 1ml Mag-Bind Particles Solution. Mix throughly by vortexing before use.

Sample Preparation

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 210 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. Centrifuge at ≥20,000 x g for 10 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 10,000 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 96-well deep well plate**. Cool the sample to room temperature by incubation at room temperature for 5 minutes.

DNA Binding

8. **Add 290µl of Mag-Bind Particles/Ethanol solution to each samples**. Mix gently by pipetting up and down 20 times.

9. **Transfer 310 µl of the sample into a 96-well round bottom plate**. Place the plate on a magnetic separation device to magnetize the magnetic particles. Sit for 5-10 minutes or until magnetic particles are fully pelleted.

   Note: If MSD-01 is used, the magnetic particles should be pelleted at the corner of each well to the magnet.

10. **Place the plate on a magnetic separation device to magnetize the magnetic particles. Sit for 5-10 minutes or until magnetic particles are fully pelleted.**

11. **Remove and discard the cleared supernatant.**

12. **Repeat step 9-11 until remaining sample are completely transferred into the Binding Plate.** Completely remove any droplets of liquid from the wall of the each well with the pipettor.

Wash the Magnetic Particles

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

14. Resuspend the magnetic particle pellet by pipetting up and down 20 times. Incubate at room temperature for 5 minutes. During incubation, mix the sample one time by pipetting up and down10 times.

    Note: Completely resuspension of the Magnetic® particles pellet is critical to obtain good results.

15. **Place the tube or microplate on a magnetic separation device to magnetize the magnetic particles.** Sample will be cleared when the magnetic particles have completely moved toward the magnet.

16. **Completely remove and discard the cleared supernatant. Let the tube and plate for 2 min. Remove any droplets of liquid from the wall of the tube or well with the pipettor.**

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

18. **Resuspend the magnetic particle pellet by pipetting up and down 20 times. Incubate for 1 minutes at room temperature.**

19. **Place the plate onto the magnetic separation device to magnetize the magnetic**

particles.

20. **Completely remove and discard the cleared supernatant. Do not disturb themagnetic Particles**.

21. **Remove and discard the cleared supernatant by pipetting.**

22. **Optional: Add 400 µl absolute ethanol and resuspend the magnetic particles by pipetting up and down 20 times.** Magnetize the magnetic particles and then aspirate the supernatant

23. **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

24. Remove the plate from the magnetic separation device. **Add 100-200 ul Elution Buffer or water to elute DNA from the magnetic particles.** Resuspend the magnetic particles by pipetting up and down 50 times.

25. **Incubate 5 minutes at 65°C.**

26. **Place the tube onto a magnetic separation device to magnetize the magnetic particles**.

27. **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).
	Ethanol is not added into MP buffer	Make sure to add ethanol in the MP Buffer (see page 4 for instruction).
Magnetic beads left-over in the solution after the beads collection	Too short of magnetizing time	Increase beads collection time on the magnet
Gelly-like material in the eluted DNA solution	Blood is too old	Remove the gelly material by centrifugation. Making sure to use fresh blood
		Use 8mM NaOH as elution buffer
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.