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Introduction

The E.Z.N.A.® Mag-Binds® Tissue DNA Kit provides a rapid and easy method for the isolation of genomic DNA for consistent PCR and Southern analysis. Up to 10 mg tissue or up to 1 cm sections of mouse tail can be readily processed in one time. The method can also be used for preparation of genomic DNA from mouse tail snips, blood, buffy coat, serum, and plasma. The kit allows single or multiple, simultaneous processing of samples. There is no need for phenol/chloroform extractions and time-consuming steps such as precipitation with isopropanol or ethanol are eliminated. The Kit allows single or multiple simultaneous processing of samples in under 1 hours. DNA purified using The E.Z.N.A.® Mag-Binds® Tissue DNA Kit is ready for applications such as PCR, Southern blotting and restriction digestion.

Overview

If using the E.Z.N.A.® Mag-Binds® Tissue DNA Kit for the first time, please read this booklet in its entirety to become familiar with the procedures. Tissue samples lysed in a specially formulated buffer. The binding conditions are adjusted so that genomic DNA will selectively bind to the Mag-Binds®particles. Two rapid wash steps remove trace contaminants such as residual polysaccharides, and pure DNA is eluted in Elution Buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

Most components of the E.Z.N.A.® Mag-Binds® Tissue DNA Kit, except RNase A, OB Protease are stable for at least 24 months from date of purchase when stored at 22°C-25°C. Mag-Bind® Particles Solution C should be stored at 4° C for long-term use. Store RNase A at -20° C. Once reconstituted in water, OB Protease should be stored at -20°C. During shipment or storage in cool ambient conditions, precipitates may form in Buffer MSL. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Kit Contents

Product Number	M6223-00	M6223-01	M6223-02
Purifications Times	5 Preps	50 Preps	200 Preps
Mag-Binds® Particles Solution C	150 µl	1.2 ml	4.5 ml
Buffer TL	5 ml	15 ml	50 ml
Buffer MSL	5 ml	20 ml	60 ml
Buffer MP	4 ml	20 ml	40 ml
OB Protease	2 mg	20 mg	4 x 20 mg
RNase A	12µl	120µl	440µl
Elution Buffer	600µl	10 ml	40 ml
Instruction Booklet	1	1	1

Before Starting

- Please read this booklet thoroughly to become familiar with the E.Z.N.A.® Mag-Bind® Tissue DNA Kit procedures.
- Equilibrate Elution Buffer (or sterile dH_2O water or 10 mM Tris pH 9.0) at 65°C.
- Dilute Buffer MP with absolute ethanol as follows and **store at room temperature**.

M6223-00	Add 6 ml absolute (96%-100%) ethanol.
M6223-01	Add 30 ml absolute (96%-100%) ethanol
M6223-02	Add 60 ml absolute (96%-100%) ethanol

- Prepare OB protease stock solution as following:

D6223-00	Add 110µl Elution Buffer to the vial
D6223-01	Add 1.05 ml Elution Buffer to the vial
D6223-02	Add 1.05 ml Elution Buffer to each vial

Vortex vial briefly prior to use. We recommend that you aliquot and store vials of reconstituted protease at -20oc.

E.Z.N.A.® Mag-Bind® Tissue DNA Kit Magnetic Protocol

Materials to be provided by user:

- Nuclease-free 1.5 ml centrifuge tube
- Water bath preset at 65°C and 55°C
- Absolute (96%-100%) ethanol
- Magnetic separation strand

NOTE: This method allows genomic DNA isolation from up to 10 mg tissue. Yields vary depending on source.

OPTIONAL: Although no mechanical homogenization of tissue is necessary, pulverizing the samples in liquid nitrogen will improve lysis and reduce incubation time. Once the liquid nitrogen has evaporated, transfer the powdered tissue to a clean 1.5 ml tube. Add 180 µl Buffer TL and 20 µl OB Protease, proceed to step 2 below.

1. **Mince 5-10 mg of tissue and place into a 1.5 ml microfuge tube. Add 180 µl Buffer TL and 20 µl OB Protease. Vortex to mix well.** Cut the tissue into small pieces to speed up lysis.

2. **Incubate at 55°C in a shaking waterbath to effect complete lysis**. If no shaking waterbath is available, vortex the sample every 20-30 minutes. Lysis time depends on amount and type of tissue, but is usually under 3 hours. One can allow lysis to proceed overnight.

   Optional: Some tissue may contains some particles than can not be digested with proteinase, briefly spin at 10,000 x g to remove those particles. Transfer the cleared lysated into a new tube or plate.

3. Add 5 µl RNase A and incubate at room temperature for 10-20 minutes.

4. **Add 220 µl Buffer MSL and vortex to mix. Incubate at 70°C for 10 minutes.**

5. Add 20 µl Mag-Bind® particles solution C and 300µl absolute ethanol (room temperature, 96-100%) to the lysate. Mix the sample gently by rotate ,shaking or pipetting up and down 20-30 times to mix well. Incubate the sample at room temperature for 5 minutes.

6. Place the tube on a magnetic separation device suitable for 1.5 ml tube to magnetize the Mag-Bind® particles for 15 min.

7. Remove and discard the cleared supernatant.
   Tip: To ensure that all traces of the supernatant are removed, let the tube

sit 2 min at room temperature and remove the remaining liquid by pipettor or Invert the tube on a paper towel for 1 min to allow residual liquid to drain.

8. Remove the tube containing the Mag-Bind®particles from the magnetic separation device. Add 400µl Buffer MP3 **diluted with ethanol** into the tube.

   Note: Buffer MP3 must be diluted with absolute ethanol (96-100%) before use in this protocol.

9. Resuspend Mag-Bind® particles pellet by pipetting up and down 20 times.

10. Place the plate onto a magnetic separation device to magnetize the Mag-Bind® particles for 10 min. Remove and discard the cleared supernatant.

11. Add 400µl of 70% ethanol and pellet by pipetting up and down 10 times. Incubate 3 min.

12. Remove and discard the cleared supernatant.

13. Add 400µl of 70% ethanol into the tube again. Incubate 1 min.

14. Remove and discard the cleared supernatant.

15. Leave the tube to air dry on the magnetic separation device for 5 minutes. Remove any residue liquid from tube by pipetting.

16. Remove the tube from magnetic separation device. Add 50-100µl Elution Buffer preheated at 70°Cor water to elute DNA from the magnetic particles.

17. Resuspend Mag-Bind® particles by pipetting up and down 20 times. Incubate 5-10 minutes at room temperature. Repeating the mix by pipetting up and down 20 times.

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

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

E.Z.N.A.® Mag-Bind® Tissue DNA Kit for Cultured cells:

This protocol is designed for the rapid isolation of up to 10 ug genomic DNA from up to 1 x 10^6 cultured cells.

1. Prepare the cell suspension
   1a. Frozen cell samples should be thawed before starting this protocol. Pellet the cells by centrifugation, wash the cells with PBS and resuspend cells with 180µl cold (4°C) PBS. Proceed with step 2 of this protocol.

   1b. For cells grown in suspension, pellet 5 x 10^6 cells by spinning at 1200 x g in a centrifuge tube. Discard the supernatant, and wash the cells once with PBS, and resuspend cells with 200µl cold (4°C) PBS.

   1c. For cells grown in a monolayer, harvest the cell by either using a trypsin treatment or scrape with rubber policemen. Wash cells twice, and resuspend the cells with 180µl cold (4°C) PBS.

2. **Add 200 ul Buffer MSL and vortex to mix well.**

3. Add 5µl RNase A and incubate at room temperature for 10 min. Proceed with the tissue protocol.

4. **Add 20 µl OB Protease and vortex to mix. Incubate at 65°C for 15 min.**

5. **Add 20 µl Mag-Bind® particles solution C and 300µl absolute ethanol (room temperature, 96-100%) to the lysate.** Mix the sample gently by rotate ,shaking or pipetting up and down 20-30 times to mix well. Incubate the sample at room temperature for 5 minutes.

6. Place the tube on a magnetic separation device suitable for 1.5 ml tube to magnetize the Mag-Bind® particles for 15 min.

7. Remove and discard the cleared supernatant.
   Tip: To ensure that all traces of the supernatant are removed, let the tube sit 2 min at room temperature and remove the remaining liquid by pipettor or Invert the tube on a paper towel for 1 min to allow residual liquid to drain.

8. Remove the tube containing the Mag-Bind®particles from the magnetic separation device. Add 400µl Buffer MP3 **diluted with ethanol** into the tube.
   Note: Buffer MP3 must be diluted with absolute ethanol (96-100%) before use in this protocol.

9. Resuspend Mag-Bind® particles pellet by pipetting up and down 20 times.

10. Place the plate onto a magnetic separation device to magnetize the Mag-Bind® particles for 10 min. Remove and discard the cleared supernatant.

11. Add 400µl of 70% ethanol and pellet by pipetting up and down 10 times. Incubate 3 min.

12. Remove and discard the cleared supernatant.

13. Add 400µl of 70% ethanol into the tube again. Incubate 1 min.

14. Remove and discard the cleared supernatant.

15. Leave the tube to air dry on the magnetic separation device for 5 minutes. Remove any residue liquid from tube by pipetting.

16. Remove the tube from magnetic separation device. Add 50-100µl Elution Buffer or water to elute DNA from the magnetic particles.

17. Resuspend Mag-Bind® particles by pipetting up and down 20 times. Incubate 5-10 minutes at room temperature. Repeating the mix by pipetting up and down 20 times.

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

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

E.Z.N.A.® Mag-Bind® Tissue DNA Kit for Mouse Tail Snips

1. **Snip two pieces of mouse tail 0.1 - 0.2 cm in length, place into a sterile 1.5 ml microcentrifuge tube, and add 180 µl of Buffer TL**. If necessary cauterize the wound to stop bleeding. Having appropriately earmarked the animal, return it to a clean cage.
   Note: Mice should not be older that 6 weeks, since lysis will be more difficult resulting in suboptimal DNA yields. If possible, obtain tail biopsy at 2-4 weeks and freeze samples at - 70°C until DNA is extracted.

2. **Add 20 µl of OB protease and vortex to mix. Incubate in a 55°C shaking waterbath for 1-4 hours or until lysis is complete.** If no shaking waterbath is available, vortex vigorously every 20-30 min. Incomplete lysis may significantly reduce DNA yields. Incubation time for complete tail lysis is dependent on length of tail and age of animal; 0.2 cm tail pieces from 2 week-old mice typically lyse in approximately 2 hours. For older animals an overnight incubation may improve yields. Note that bone and hair will not lyse.

3. To remove RNA, add 5 µl of RNase A and incubate 10 min at room temperature.

4. **Centrifuge for 5 min at 10,000 x g to pellet insoluble tissue debris and hair**. Carefully aspirate the supernatant and transfer to a sterile microfuge tube leaving behind any insoluble pellet.

5. **Add 220 µl Buffer MSL and vortex to mix. Incubate at 65°C for 10 minutes.**

6. Add 20µl Mag-Bind® particles solution C and 300 µl absolute ethanol (room temperature, 96-100%) to the lysate. Mix the sample gently by rotate ,shaking or pipetting up and down 20-30 times to mix well. Incubate the sample at room temperature for 5 minutes.

7. Place the tube on a magnetic separation device suitable for 1.5 ml tube to magnetize the Mag-Bind® particles for 15 min.

8. Remove and discard the cleared supernatant.
   Tip: To ensure that all traces of the supernatant are removed, let the tube sit 2 min at room temperature and remove the remaining liquid by pipettor or Invert the tube on a paper towel for 1 min to allow residual liquid to drain.

9. Remove the tube containing the Mag-Bind® particles from the magnetic separation device. Add 400µl Buffer MP3 **diluted with ethanol** into the tube.

   Note: Buffer MP3 must be diluted with absolute ethanol (96-100%) before use in this protocol.

10. Resuspend Mag-Bind® particles pellet by pipetting up and down 20 times.

11. Place the plate onto a magnetic separation device to magnetize the Mag-Bind® particles for 10 min. Remove and discard the cleared supernatant.

12. Add 400µl of 70% ethanol and pellet by pipetting up and down 10 times. Incubate 3 min.

13. Remove and discard the cleared supernatant.

14. Add 400µl of 70% ethanol into the tube again. Incubate 1 min.

15. Remove and discard the cleared supernatant.

16. Leave the tube to air dry on the magnetic separation device for 5 minutes. Remove any residue liquid from tube by pipetting.

17. Remove the tube from magnetic separation device. Add 50-100µl Elution Buffer or water to elute DNA from the magnetic particles.

18. Resuspend Mag-Bind® particles by pipetting up and down 20 times. Incubate 5-10 minutes at room temperature. Repeating the mix by pipetting up and down 20 times.

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

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

E.Z.N.A.® Mag-Bind® Tissue DNA Kit for Buccal Swabs

The following protocol is designed for isolating DNA from buccal swabs with magnetic beads.

1. Place the swabs into the tube and **add 300ul Buffer TL.**

2. **Add 20 ul OB Protease, vortex to mix throughly and incubate at 55°C in a waterbath for 45 minutes to effect complete lysis.**

3. **Add 300 ul Buffer MSL and vortex to mix througly** and incubate at 65°C in a waterbath for 10 minutes.

4. **Briefly spin and transfer 400ul of the lysate into a new tube.**

5. **Add 20µl Mag-Bind® particles solution C and 300 µl absolute ethanol (room temperature, 96-100%) to the lysate.** Mix the sample gently by rotate ,shaking or pipetting up and down 20-30 times to mix well. Incubate the sample at room temperature for 5 minutes.

6. Place the tube on a magnetic separation device suitable for 1.5 ml tube to magnetize the Mag-Bind® particles for 15 min.

7. Remove and discard the cleared supernatant.
   Tip: To ensure that all traces of the supernatant are removed, let the tube sit 2 min at room temperature and remove the remaining liquid by pipettor or Invert the tube on a paper towel for 1 min to allow residual liquid to drain.

8. **Add 400µl of 70% ethanol** and pellet by pipetting up and down 10 times. Incubate 3 min.

9. Remove and discard the cleared supernatant.

10. **Add 400µl of 70% ethanol into the tube again**. Incubate 1 min.

11. Remove and discard the cleared supernatant.

12. Leave the tube to air dry on the magnetic separation device for 5 minutes. Remove any residue liquid from tube by pipetting.

13. Remove the tube from magnetic separation device. Add 50-100µl Elution Buffer or water to elute DNA from the magnetic particles.

14. Resuspend Mag-Bind® particles by pipetting up and down 20 times. Incubate 5-10 minutes at room temperature. Repeating the mix by pipetting up and down 20 times.

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

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

E.Z.N.A.® Mag-Bind® Tissue Protocol for Paraffin-Embedded Tissue

1. **Place not more than 10 mg tissue (2-5 of ~20um sections) in a new 2 ml tube.** Extract the sample with 1 ml xylene to remove the paraffin. Mix thoroughly by vortexing.

2. Centrifuge the tube at 10,000 x g for 10 min at room temperature. Discard supernatant without disturbing the tissue pellet.

3. Rinse the pellet with 1 ml absolute ethanol to remove traces of xylene. Centrifuge at 10,000 x g for 5 min at room temperature. Discard the ethanol without disturbing the tissue pellet.

4. Repeat the ethanol rinse.

5. Air dry tissue pellet at 37°C for 15 min.

6. Add 180 µl Buffer TL and 20ul OB Proteaseto the tissue and **Vortex to mix well.**

7. **Incubate at 55°C in a shaking waterbath for overnight to effect complete lysis**.

8. Incubate at 90°C for 1 hour. Centrifuge at 10,000xg for 5 minutes to remove the undigested particles. Transfer the supernatant into a new tube.

9. **Add 220µl Buffer MSL**, **20µl Mag-Bind® particles solution C and 300 µl absolute ethanol (room temperature, 96-100%) to the lysate.** Mix the sample gently by rotate ,shaking or pipetting up and down 20-30 times to mix well. Incubate the sample at room temperature for 5 minutes.

10. Place the tube on a magnetic separation device suitable for 1.5 ml tube to magnetize the Mag-Bind® particles for 15 min.

11. Remove and discard the cleared supernatant.
    Tip: To ensure that all traces of the supernatant are removed, let the tube sit 2 min at room temperature and remove the remaining liquid by pipettor or Invert the tube on a paper towel for 1 min to allow residual liquid to drain.

12. **Add 400µl of 70% ethanol** and pellet by pipetting up and down 10 times. Incubate 3 min.

13. Remove and discard the cleared supernatant.

14. **Add 400µl of 70% ethanol into the tube again**. Incubate 1 min.

15. Remove and discard the cleared supernatant.

16. Leave the tube to air dry on the magnetic separation device for 5 minutes. Remove any residue liquid from tube by pipetting.

17. Remove the tube from magnetic separation device. Add 50-100µl Elution Buffer or water to elute DNA from the magnetic particles.

18. Resuspend Mag-Bind® particles by pipetting up and down 20 times. Incubate 5-10 minutes at room temperature. Repeating the mix by pipetting up and down 20 times.

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.

Note: Tissue fixed with paraformaldehyde will yield degraded DNA or RNA. The extent of degradation depends on type of fixative used, but the size of DNA obtained is usually less than 500 bp. Degradation is not caused by the E.Z.N.A.® Tissue DNA protocol, and for PCR detection of segments smaller than 500 bp satisfactory results can be obtained.

Troubleshooting Guide

Problem	Likely Cause	Suggestions
Low DNA yield	Incomplete resuspension of magnetic particle	Resuspend the magnetic particles by vortexing before use.
	Frozen Tissue samples not mixed properly after thawing.	Thaw the frozen Tissue at room temperature an d gently mix the Tissue by inverting.
	Loss the Mag-Bind® particle during operation	Carefully avoid remove the Mag-Bind® particles during aspiration
	DNA remains bound to Mag-Bind®Particles	Increase elution volume and incubate at 65oC for 5 min elution. Pipet up and down for 50-100 times.
Problemsin downstream applications	Ethanol carry-over	Dry the Mag-Bind particle before elution.