Contents

Revised May 2008

Introduction

The E-Z 96® 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 96® Mag-Binds® Tissue DNA Kit is ready for applications such as PCR, Southern blotting and restriction digestion.

Overview

If using the E-Z 96® 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 96® 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, proteinase K should be stored at -20°C. During shipment or storage in cool ambient conditions, precipitates may form in Buffer MSL and Buffer TL. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Kit Contents

Product Number	M6229-00	M6229-01	M6229-02
Purification Times	1 x 96	4 x 96	20 x 96
500 µl Process Plate	1	4	20
Racked Microtubes (1.2 mL)	1	4	20
8-Strip Microtube Caps	24 x 8	100 x 8	500 x 8
96 Microplate (300µL)	1	4	20
Mag-Bind Particles Solution F	2.2 ml	8.2 ml	41ml
Buffer TL	20 ml	80 ml	350 ml
Buffer MSL	20 ml	80 ml	350 ml
Buffer MP	20 ml	80 ml	400 ml
Proteinase K	40 mg	160 mg	4 x 160
RNase A	550 µl	2.1 ml	10 ml
Elution Buffer	30 ml	100 ml	400 ml
Instruction Booklet	1	1	1

Before Starting

- Please read this booklet thoroughly to become familiar with the E-Z 96® 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**.

M6229-00	Add 30 ml absolute (96%-100%) ethanol
M6229-01	Add 120 ml absolute (96%-100%) ethanol
M6229-02	Add 600 ml absolute (96%-100%) ethanol

- Prepare an Proteinase K stock solution at 20 mg/mL with DNA Elution Buffer and aliquot into adequate portions. Store each aliquot at -20°C and thaw before use. Each sample will require 20 µl of this solution.

D6229-00	Add 2 ml Elution Buffer to the vial
D6229-01	Add 8 ml Elution Buffer to the vial
D6229-02	Add 8 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 96® Mag-Bind Tissue DNA Protocol for Tissues

Materials to be provided by user:

- Laboratory centrifuge capable of 3,000-5,000 x g equipped with swinging-bucket rotor.
- Adapter for deep-well microplate
- Waterbath equilibrated to 56°C and 65°C
- Equilibrated sterile dH_2O water or Elution Buffer at 65°C
- Absolute (96%-100%) ethanol
- Multichannel pipet with tips
- E-Z 96 Magnetic Separation Strand **(Cat# MSD-01)**

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 Racked Microtubes. Add 150 µl Buffer TL/OB Protease and proceed to step 3 below.

1. **Mince 5-10 mg tissues and place into each well of Racked Microtubes.** Make a chart to record the position of each sample.

2. **Prepare an OB Protease/Buffer TL working solution by mixing 20 µl Proteinase K with 130 ul Buffer TL for each sample.** For each 96 sets of samples, prepare the Protease/TL stock working solution by mix 2.0 mL protease with 13 mL Buffer TL. Pipet 150 µl protease/TL working solution into each well. Seal the plate properly using the caps supplied.

3. **Mix the samples by inverting the plate 3-5 times.** Briefly spin the plate at 2,500 -3,000 x g to collect any residue solution from the caps. It is very important that samples are completely submerged in the solution. If the protease/TL solution does not completely cover the sample, increase the sample volume to 200µl. (Additional reagent can be purchased separately).

4. **Incubate at 56°C overnight or until the samples are completely lysed.** The lysate should be clear and viscous after digestion is complete. Mix occasionally during the incubation by rotating the plate gently. Make sure the samples are completely lysed.

5. **Shake or vortex the plate vigorously from side to side.** (Do not shake up and down to avoid leaking around the caps.) Hold the caps to ensure the plate is sealed properly. Ensure the lysate is completely homogenous after shaking. If a gelatinous mass is visible, further digestion is required.

6. **Optional:** Certain tissues such as liver have high levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point. For isolation of RNA-Free genomic DNA, spin briefly to collect any drops and add 5µl RNase A solution (20mg/mL) to each sample and incubate 10-20 minutes to remove the RNA.

7. **Remove the caps and add 150 ul Buffer MSL to each sample.** A white precipitate may form at this step; it will not interfere with DNA isolation. Seal the plate with new caps (supplied).

8. **Mix the sample by shaking or vortex the plate vigorously (side to side) for 1 minute.** Spin briefly to collect any liquid from the caps (the centrifugation speed should be less than 3000 x g).

9. Incubate at 65°C for 10 min.

10. **Optional: Spin at 3,000-5,000 x g for 10 minutes at room temperature to remove undigested particles.**

11. **Remove the caps and transfer 200 µl of the supernatant into each well of 500 µl Process Plate (provided).**

12. **Add 20 µl Mag-Bind® particles solution F and 160 µl absolute ethanol (room temperature, 96-100%) to the lysate.** Mix the sample by pipetting up and down 20-30 times to mix well. Incubate the sample at room temperature for 5 minutes.

13. **Place the Plate on a magnetic separation device (MSD-01) to magnetize the Mag-Bind® particles for 10-15 min.** The liquid should be cleared after all the magnetic beads are pelleted on the corner of the each well adjecent to the magnet.

14. **Aspirate and discard the cleared supernatant**.

15. Remove the Plate containing the Mag-Bind®particles from the magnetic separation device. Add 400µl Buffer MP3 **diluted with ethanol** into each well.

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

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

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

18. **Remove the plate from the magnetic separation device. Add 400µl of 70% ethanol and pellet by pipetting up and down 5 times.** Incubate 2 min.

19. Aspirate and discard the cleared supernatant.

20. **Add 400µl of 70% ethanol into the tube again.** Incubate 2 min.

21. Remove and discard the cleared supernatant.

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

23. Remove the Plate from magnetic separation device. **Add 50-100µl Elution Buffer preheated at 70°C to elute DNA from the magnetic particles.**

24. **Resuspend Mag-Bind® particles by pipetting up and down 20-30 times.** Incubate 5 minutes at 37C or 15 minutes at room temperature.

25. **Place the plate onto magnetic separation device to magnetize the Mag-Bead® particles.** Wait 7–10 minutes or until magnetic beads are cleared from the solution.

26. **Transfer the cleared supernatant containing purified DNA to a new 300µl microplate (suplied).**

27. **Seal the plate with sealing film store at -20°C.**

E-Z 96® 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 a RNase/PBS working solution by mixing 5 µl RNase A with 100ul PBS for each sample.** For each 96 sets of sample, prepare the Rnase/PBS stock working solution by mix 0.5 mL RNase with 10 mL PBS.

2. **Prepare the cell suspension**
   2a. Frozen cell samples should be thawed before starting this protocol. Pellet the cells by centrifugation, wash the cells with PBS and resuspend cells with 100µl cold (4°C) PBS/RNase. Proceed with step 2 of this protocol.

   2b. For cells grown in suspension, pellet 1 x 10^6 cells by spinning at 1200 x g. Discard the supernatant and wash the cells once with PBS. Resuspend cells with 100µl cold (4°C) PBS/RNase.

   2c. 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 100µl cold (4°C) PBS/RNase.

3. **Transfer the suspended cells into each well of Racked Microtubes.** Make a chart to record the position of each sample.

4. **Add 120 Buffer MSL into each well of Racked Microtubes**. Seal the plate properly using the caps supplied.

5. **Mix the samples by inverting the plate.** Briefly spin the plate at 2,500 - 3,000 x g to collect any residue solution from the caps. Incubate at room temperature for 10 minutes to digest RNA.

6. **Remove the caps and add 20 µl Proteinase K into each well of Racked Microtubes.**

7. **Seal the plate properly using the new caps supplied. Mix the samples by shaking or vortexing.** Briefly spin the plate at 2,500 -3,000 x g to collect any residue solution from the caps. **Incubate at 65°C for 15 min.**

8. Remove the caps and transfer 200ul of the supernatant into a new 500ul Process Plate (provided). Following step 12-27 on page 5-7.

E-Z 96 Mag-Bind® Tissue DNA Kit for Mouse Tail Snips

1. **Snip one piece of mouse tail 0.1 - 0.2 cm in length, place into a new Racked Microtubes.** If necessary cauterize the wound to stop bleeding.

   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. **Prepare a Proteinase K/Buffer TL working solution by mixing 20 µl Proteinase K with 130ul Buffer TL for each sample.** For each 96 sets of sample, prepare the Protease/TL stock working solution by mix 2.0mL protease with 13 mL Buffer TL. Pipet 150 µl protease/TL working solution into each well. Seal the plate properly using the caps supplied.

3. **Mix the samples by inverting the plate.** Briefly spin the plate at 2,500 - 3,000 x g to collect any residue solution from the caps. It is very important that samples are completely submerged in the solution. If the protease/TL solution does not completely cover the sample, increase the sample volume to 200µl. (Additional reagent can be purchased separately).

4. **Incubate at 56°C over night or until the samples are completely lysed.** The lysate should be clear and viscous after digestion is complete. Mix occasionally during the incubation by rotating the plate gently. Make sure the samples are completely lysed.

5. **Shake or vortex the plate vigorously from side to side.** (Do not shake up and down to avoid leaking around the caps.) Hold the caps to ensure the plate is sealed properly. Ensure the lysate is completely homogenous after shaking. If a gelatinous mass is visible, further digestion is required.

6. **Optional: For isolation of RNA-Free genomic DNA, spin briefly to collect any drops and add 5µl RNase A solution (20mg/mL) to each sample** and incubate 10-20 minutes to remove the RNA.

7. **Remove the caps and add 150 ul Buffer MSL to each sample.** A white precipitate may form at this step; it will not interfere with DNA isolation. Seal the plate with new caps (supplied).

8. Mix the sample by shaking or vortex the plate vigorously (side to side) for 1 minute. Spin briefly to collect any liquid from the caps (the centrifugation speed should be less than 3000 x g).

9. **Incubate at 65°C for 10 min.**

10. **Spin at 3,000-5,000 x g for 10 minutes at room temperature to remove undigested particles.**

11. **Transfer 200 ul of the supernatant into each well of 500ul Process Plate.**

12. Following step 12-27 on page 5-7.

E-Z 96® 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 swab into each well of Racked Microtubes.**

2. **Prepare an Proteinase K/Buffer TL/MSL working solution by mixing 20 µl Proteinase K with 200 µl Buffer TL and 200 µl Buffer MSL for each sample.** For each 96 sets of sample, prepare the Protease/TL / MSL stock working solution by mix 2 mL proteinase with 20 ml Buffer TL and 20ml Buffer MSL. Pipet 420 µl stock working solution into each well. Seal the tube using the strip caps (supplied).

3. **Mix the samples by inverting the plate.** Briefly spin the plate at 2,500 - 3,000 x g to collect any residue solution from the caps. It is very important that samples are completely submerged in the solution. If the protease/TL/MSL solution does not completely cover the sample, increase the sample volume to 600µl. (Additional reagent can be purchased separately).

4. **Incubate at 56°C for 1-3 hours.** Mix occasionally during the incubation by rotating the plate gently. Make sure the samples are completely lysed.

5. **Shake or vortex the plate vigorously from side to side.** (Do not shake up and down to avoid leaking around the caps.) Hold the caps to ensure the plate is sealed properly. Ensure the lysate is completely homogenous after shaking. If a gelatinous mass is visible, further digestion is required.

6. **Spin at 3,000-5,000 x g for 10 minutes at room temperature to remove the swab.**

7. **Remove the caps and transfer 200 µl of the supernatant into each well of 500ul Process Plate (provided).**

8. **Following step 12-27 on page 5-7.**

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.
Problem in downstream applications	Ethanol carry-over	Dry the Mag-Bind particle before elution.