OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

Mag-Bind® Tissue DNA Kit

M6223-00	5 preps
M6223-02	200 preps

Mag-Bind® Tissue DNA 96 Kit

M6229-00	1 x 96 preps
M6229-01	4 x 96 preps

June 2013

For research use only.Not intended for diagnostic testing.

Mag-Bind® Tissue DNA Kit
Mag-Bind® Tissue DNA 96 Kit

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Introduction and Overview

The Mag-Bind® Tissue DNA Kit and the Mag-Bind® Tissue DNA 96 Kit are designed for rapid and reliable isolation of high-quality genomic DNA from a wide variety of tissues and cultured cells. Up to 20 mg animal tissue (1.5 mL microcentrifuge tube format), 10 mg animal tissue (96-well deep-well plate format), or 5 x 10^6 cells can be processed in less than 1 hour. The Mag-Bind® paramagnetic particles technology provides high-quality DNA which is suitable for direct use in most downstream applications such as amplifications and enzymatic reactions. This system can be easily adapted to a variety of automated platforms and the procedure can be scaled up or down, allowing for purification from various amounts of starting material.

If using the Mag-Bind® Tissue DNA Kit or the Mag-Bind® Tissue DNA 96 Kit for the first time, please read this booklet to become familiar with the procedure and its various modifications. Animal tissue or cultured cells are pretreated with Proteinase K and lysed in a specially formulated buffer containing detergent. DNA binds to the surface of Mag-Bind® Particles SC. Proteins, polysaccharides, and cellular debris are efficiently washed with three wash steps. Pure DNA is eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

New in this Edition: This manual has been edited for content and redesigned to enhance user readability.

- Proteinase K is now supplied in a liquid form eliminating the step to resuspend prior to use.
- Proteinase K Solution can be stored at room temperature for 12 months.
- Proteinase Storage Buffer is no longer included in the kit.

Kit Contents

Mag-Bind® Tissue DNA Kit	M6223-00	M6223-02
Purifications	5	200
Mag-Bind® Particles SC	55 µL	2.2 mL
MSL Buffer	1.5 mL	60 mL
TL Buffer	3 mL	120 mL
MP Buffer	2 mL	40 mL
SPM Wash Buffer	3 mL	60 mL
LPA Buffer	55 µL	2.2 mL
Elution Buffer	2 mL	120 mL
RNase A	30 µL	650 µL
Proteinase K Solution	150 µL	6 mL
User Manual	✓	✓

Mag-Bind® Tissue DNA 96 Kit	M6229-00	M6229-01
Purifications	1 x 96	4 x 96
Mag-Bind® Particles SC	1.1 mL	4.4 mL
MSL Buffer	33 mL	125 mL
TL Buffer	30 mL	120 mL
MP Buffer	25 mL	100 mL
SPM Wash Buffer	30 mL	2 x 75 mL
LPA Buffer	1.1 mL	4.4 mL
Elution Buffer	30 mL	120 mL
RNase A	1 mL	4 mL
Proteinase K Solution	2.5 mL	10 mL
User Manual	✓	✓

Storage and Stability

Most components of the Mag-Bind® Tissue DNA Kit and the Mag-Bind® Tissue DNA 96 Kit can be stored at room temperature except the Mag-Bind® Particles SC and LPA Buffer. Mag-Bind® Particles SC and LPA Buffer must be stored at 2-8°C. Proteinase K Solution can be stored at room temperature for 12 months. For long-term storage (>12 months), store Proteinase K Solution at 2-8°C. Under these conditions, performance of all components of the kit are guaranteed at least 12 months. Under cool ambient conditions, a precipitate may form in the MSL Buffer and TL Buffer. In case of such an event, heat the bottle to 37°C to dissolve the precipitate.

Preparing Reagents

1. Dilute SPM Wash Buffer with 100% ethanol as follows and store at room temperature.

Product	100% Ethanol to be Added
M6223-00	7 mL
M6223-02	140 mL
Product	**100% Ethanol to be Added**
M6229-00	70 mL
M6229-01	175 mL per bottle

2. Dilute MP Buffer with 100% ethanol as follows and store at room temperature.

Product	100% Ethanol to be Added
M6223-00	3 mL
M6223-02	60mL
Product	**100% Ethanol to be Added**
M6229-00	37.5 mL
M6229-01	150 mL

Mag-Bind® Tissue DNA Kit Protocol - Tissue

This method allows genomic DNA isolation from up to 20 mg tissue. Yields vary depending on the source.

Materials and Equipment to be Supplied by User:

- Magnetic separation device for 1.5 mL microcentrifuge tubes (Cat# MSD-02)
- Tabletop microcentrifuge
- Nuclease-free 1.5 mL microcentrifuge tubes
- Water bath, incubator, or heat block capable of 70°C
- Shaking water bath capable of 55°C
- Vortexer
- 100% ethanol

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4.
- Preheat Elution Buffer to 70°C.
- Set shaking water bath to 55°C.

OPTIONAL: Although mechanical homogenization of tissue is not 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 microcentrifuge tube. Add 200 µL TL Buffer and proceed to Step 2 below.

1. Mince up to 20 mg tissue and place into a 1.5 mL microcentrifuge tube. Add 250 µL TL Buffer.

   Note: Cutting the tissue into small pieces can speed up lysis.

2. Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.

3. Incubate at 55°C in a shaking water bath. Lysis time will depend on the amount and type of tissue, but is usually under 3 hours. Lysis can proceed overnight.

   Note: If a shaking water bath is not available, vortex the sample every 20-30 minutes.

4. Centrifuge at 13,000 x *g* for 5 minutes.

5. Carefully transfer 200 µL of the cleared supernatant to a new 1.5 mL microcentrifuge tube.

6. Add 200 µL MSL Buffer and 5 µL RNase A. Vortex or pipet up and down to mix thoroughly.

7. Incubate at 70°C for 10 minutes.

8. Place the sample on the bench for 5 minutes to bring the sample to room temperature.

9. Add 290 µL 100% ethanol and 10 µL Mag-Bind® Particles SC. Vortex or pipet up and down 20-30 times to mix thoroughly.

10. Let sit at room temperature for 5 minutes.

    Optional: For samples with an expected yield less than 8 µg, add 10 µL LPA Buffer to help increase the yield.

11. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit for 10-15 minutes.

12. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

13. Remove the tube from the magnetic separation device.

14. Add 400 µL MP Buffer.

    Note: MP Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

15. Resuspend the Mag-Bind® Particles SC by vortexing or pipetting up and down 20-30 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles SC.

16. Let sit for 3 minutes at room temperature. Vortex or pipet up and down a few times during incubation.

17. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

18. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

19. Remove the tube from the magnetic separation device.

20. Add 400 µL SPM Wash Buffer.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

21. Resuspend the Mag-Bind® Particles SC by vortexing or pipetting up and down 20-30 times.

22. Let sit for 3 minutes at room temperature. Vortex or pipette up and down a few times during incubation.

23. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

24. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

25. Repeat Steps 19-24 for a second SPM Wash Buffer wash step.

26. Leave the tube on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles SC. Remove any residual liquid with a pipettor.

27. Remove the tube from the magnetic separation device.

28. Add 50-200 µL Elution Buffer preheated to 70°C.

29. Resuspend the Mag-Bind® Particles SC by vortexing for 3 minutes or pipetting up and down 50 times.

30. Let sit at room temperature for 5-10 minutes.

31. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

32. Transfer the cleared supernatant containing purified DNA to a nuclease-free 1.5 mL microcentrifuge tube.

    Note: The expected yield from a 20 mg sample is 8-35 µg genomic DNA depending on type of tissue.

33. Store DNA at -20°C.

Mag-Bind® Tissue DNA Kit Protocol - Cultured Cells

This protocol is designed for rapid isolation of up to 25 µg genomic DNA from up to 5 x 10^6 cultured cells.

Materials and Equipment to be Supplied by User:

- Magnetic separation device for 1.5 mL microcentrifuge tubes (Cat# MSD-02)
- Tabletop microcentrifuge
- Nuclease-free 1.5 mL microcentrifuge tubes
- Water bath, incubator, or heat block capable of 70°C
- Shaking water bath capable of 55°C
- Vortexer
- 100% ethanol
- Cold PBS (4°C)

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4.
- Preheat Elution Buffer to 70°C.
- Set shaking water bath to 55°C.

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 cold PBS (4°C), and resuspend cells in 180 µL cold PBS. Proceed with Step 2 of this protocol.

   1b. For cells grown in suspension, pellet 5 x 10^6 cells at 1,200 x *g* in a microcentrifuge tube. Discard the supernatant and wash the cells once with cold PBS (4°C). Resuspend cells in 180 µL cold PBS. Proceed with Step 2 of this protocol.

   1c. For cells grown in a monolayer, harvest the cells by either using a trypsin treatment or cell scraper. Wash cells twice with cold PBS (4°C) and resuspend the cells in 180 µL cold PBS. Proceed with Step 2 of this protocol.

2. Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.

3. Incubate at 55°C for 10 minutes in a shaking water bath.

   Note: If a shaking water bath is not available, vortex the sample every 2-3 minutes.

4. Transfer the sample to a new 1.5 mL microcentrifuge tube.

5. Add 200 µL MSL Buffer. Vortex or pipet up and down to mix thoroughly.

6. Incubate at 70°C for 10 minutes.

7. Place the sample on the bench for 5 minutes to bring the sample to room temperature.

8. Add 260 µL 100% ethanol,10 µL Mag-Bind® Particles SC, and 10 µL LPA Buffer. Vortex or pipet up and down 20-30 times to mix thoroughly.

9. Let sit at room temperature for 5 minutes.

10. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit for 10-15 minutes.

11. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

12. Remove the tube from the magnetic separation device.

13. Add 400 µL MP Buffer.

    Note: MP Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

14. Resuspend the Mag-Bind® Particles SC by vortexing or pipetting up and down 20-30 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles SC.

15. Let sit for 3 minutes at room temperature. Vortex or pipet up and down a few times during incubation.

16. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

17. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

18. Remove the tube from the magnetic separation device.

19. Add 400 µL SPM Wash Buffer.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

20. Resuspend the Mag-Bind® Particles SC by vortexing or pipetting up and down 20-30 times.

21. Let sit for 3 minutes at room temperature. Vortex or pipet up and down a few times during incubation.

22. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

23. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

24. Repeat Steps 18-23 for a second SPM Wash Buffer wash step.

25. Leave the tube on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles SC. Remove any residual liquid with a pipettor.

26. Remove the tube from the magnetic separation device.

27. Add 50-200 µL Elution Buffer preheated to 70°C.

28. Resuspend the Mag-Bind® Particles SC by vortexing for 3 minutes or pipetting up and down 50 times.

29. Let sit at room temperature for 5-10 minutes.

30. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

31. Transfer the cleared supernatant containing purified DNA to a nuclease-free 1.5 mL microcentrifuge tube.

32. Store DNA at -20°C.

Mag-Bind® Tissue DNA Kit Protocol - Mouse Tail Snips

Materials and Equipment to be Supplied by User:

- Magnetic separation device for 1.5 mL microcentrifuge tubes (Cat# MSD-02)
- Tabletop microcentrifuge
- Nuclease-free 1.5 mL microcentrifuge tubes
- Water bath, incubator, or heat block capable of 70°C
- Shaking water bath capable of 55°C
- Vortexer
- 100% ethanol

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4
- Preheat Elution Buffer to 70°C.
- Set shaking water bath to 55°C.

1. Snip a 2-5 mm piece of mouse tail, cut into several pieces, and place the pieces into a nuclease-free 1.5 mL microcentrifuge tube.

   Note: Follow all regulations regarding the safe and humane treatment of animals. Mice should not be older than 6 weeks as lysis will be more difficult in older animals resulting in suboptimal DNA yields. If possible, obtain tail biopsies at 2-4 weeks and freeze samples at -70°C until DNA is extracted.

2. Add 250 µL TL Buffer.

3. Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.

4. Incubate at 55°C in a shaking water bath for 1-4 hours or until lysis is complete.

   Note: If a shaking water bath is not available, vortex the samples vigorously every 20-30 minutes. Incomplete lysis may significantly reduce DNA yields. Incubation time for complete tail lysis is dependent on length of tail snip and age of animal, e.g. a 5 mm tail piece from a 2 week old mouse typically will lyse in 2 hours. For older animals, an overnight incubation may improve yields. Note that bone and hair will not lyse.

5. Centrifuge at maximum speed for 5 minutes to pellet undigested tissue debris and hair. Carefully transfer 200 µL of the supernatant to a new 1.5 mL microcentrifuge tube without disturbing the undigested pellet.

OPTIONAL: Mouse tail tissue contains RNA that can be purified with the DNA. This will not interfere with PCR reactions, but other enzymatic reactions may be affected. To remove RNA, add 5 µL RNase A and let sit at room temperature for 2 minutes. Proceed to Step 6.

6. Add 200 µL MSL Buffer. Vortex or pipet up and down to mix thoroughly.

7. Incubate at 70°C for 10 minutes.

8. Place the sample on the bench for 5 minutes to bring the sample to room temperature.

9. Add 260 µL 100% ethanol,10 µL Mag-Bind® Particles SC, and 10 µL LPA Buffer. Vortex or pipet up and down 20-30 times to mix thoroughly.

10. Let sit at room temperature for 5 minutes.

11. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit for 10-15 minutes.

12. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

13. Remove the tube from the magnetic separation device.

14. Add 400 µL MP Buffer.

    Note: MP Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

15. Resuspend the Mag-Bind® Particles SC by vortexing or pipetting up and down 20-30 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles SC.

16. Let sit for 3 minutes at room temperature. Vortex or pipet up and down a few times during incubation.

17. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

18. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

19. Remove the tube from the magnetic separation device.

20. Add 400 µL SPM Wash Buffer.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

21. Resuspend the Mag-Bind® Particles SC by vortexing or pipetting up and down 20-30 times.

22. Let sit for 3 minutes at room temperature. Vortex or pipet up and down a few times during incubation.

23. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

24. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

25. Repeat Steps 19-24 for a second SPM Wash Buffer wash step.

26. Leave the tube on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles SC. Remove any residual liquid with a pipettor.

27. Remove the tube from the magnetic separation device.

28. Add 50-200 µL Elution Buffer preheated to 70°C.

29. Resuspend the Mag-Bind® Particles SC by vortexing for 3 minutes or pipetting up and down 50 times.

30. Let sit at room temperature for 5-10 minutes.

31. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

32. Transfer the cleared supernatant containing purified DNA to a nuclease-free 1.5 mL microcentrifuge tube.

33. Store DNA at -20°C.

Mag-Bind® Tissue DNA Kit Protocol - Buccal Swabs

Materials and Equipment to be Supplied by User:

- Magnetic separation device for 1.5 mL microcentrifuge tubes (Cat# MSD-02)
- Tabletop microcentrifuge
- Nuclease-free 1.5 mL microcentrifuge tubes
- Water bath, incubator, or heat block capable of 70°C
- Shaking water bath capable of 55°C
- Vortexer
- 100% ethanol

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4.
- Preheat Elution Buffer to 70°C.
- Set shaking water bath to 55°C.

1. Cut off the buccal brush or swab head and place into a 1.5 mL microcentrifuge tube.

2. Add 400 µL TL Buffer.

3. Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.

4. Incubate at 55°C in a shaking water bath for 45 minutes.

   Note: If a shaking water bath is not available, vortex the sample every 5-10 minutes.

5. Centrifuge at 3,000 x *g* for 10 minutes.

6. Transfer 200 µL lysate into a new 1.5 mL microcentrifuge tube. Do not transfer the swab to the new tube.

7. Add 200 µL MSL Buffer. Vortex or pipet up and down to mix thoroughly.

8. Incubate at 70°C for 10 minutes.

9. Place the sample on the bench for 5 minutes to bring the sample to room temperature.

10. Add 260 µL 100% ethanol,10 µL Mag-Bind® Particles SC, and 10 µL LPA Buffer. Vortex or pipet up and down 20-30 times to mix thoroughly.

11. Let sit at room temperature for 5 minutes.

12. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit for 10-15 minutes.

13. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

14. Remove the tube from the magnetic separation device.

15. Add 400 µL MP Buffer.

    Note: MP Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

16. Resuspend the Mag-Bind® Particles SC by vortexing or pipetting up and down 20-30 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles SC.

17. Let sit for 3 minutes at room temperature. Vortex or pipet up and down a few times during incubation.

18. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

19. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

20. Remove the tube from the magnetic separation device.

21. Add 400 µL SPM Wash Buffer.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

22. Resuspend the Mag-Bind® Particles SC by vortexing or pipetting up and down 20-30 times.

23. Let sit for 3 minutes at room temperature. Vortex or pipet up and down a few times during incubation.

24. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

25. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

26. Repeat Steps 20-25 for a second SPM Wash Buffer wash step.

27. Leave the tube on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles SC. Remove any residual liquid with a pipettor.

28. Remove the tube from the magnetic separation device.

29. Add 50-200 µL Elution Buffer preheated to 70°C.

30. Resuspend the Mag-Bind® Particles SC by vortexing for 3 minutes or pipetting up and down 50 times.

31. Let sit at room temperature for 5-10 minutes.

32. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

33. Transfer the cleared supernatant containing purified DNA to a nuclease-free 1.5 mL microcentrifuge tube.

34. Store DNA at -20°C.

Mag-Bind® Tissue DNA 96 Kit Protocol - Tissue

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

Materials and Equipment to be Supplied by User:

- Magnetic separation device for 96-well plates (Cat# MSD-01B or MSD-01)
- 96-well deep-well plates compatible with the magnetic separation device
- 96-well microplates
- Centrifuge with swing-bucket rotor capable of 4000 x *g*
- Centrifuge adaptor for 96-well plates
- Water bath, incubator, or heat block capable of 70°C
- Shaking water bath capable of 55°C
- Vortexer
- 100% ethanol
- Sealing film
- Multi-channel reservoir

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4.
- Preheat Elution Buffer to 70°C.
- Set water bath to 55°C.

OPTIONAL: Although mechanical homogenization of tissue is not 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 96-well deep-well plate. Add 200 µL TL Buffer and proceed to Step 3 below.

1. Mince up to 10 mg tissue and transfer to a 96-well deep-well plate.

   Note: Cutting the tissue into small pieces can speed up lysis.

2. Add 250 µL TL Buffer.

3. Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.

   Note: Seal the plate before vortexing to prevent cross-contamination or loss of sample.

4. Incubate at 55°C in a shaking water bath.

   Note: If a shaking water bath is not available, vortex the sample every 20-30 minutes. Lysis time depends on amount and type of tissue, but is usually under 3 hours. The lysis can proceed overnight.

   Important: Some tissue may contain material that can not be digested with proteinase; centrifuge the plate at maximum speed for 5 minutes to remove the undigested material. Transfer the cleared lysate to a new plate.

OPTIONAL: Certain tissues such as liver have high levels of RNA which will be purified with DNA using this kit. While it will not interfere with PCR, the RNA can be removed at this point. Add 5 µL RNase A (assuming a sample size of 10 mg) and let sit at room temperature for 2 minutes. Proceed to Step 5.

5. Centrifuge at maximum speed for 5 minutes to pellet undigested tissue debris and hair.

6. Carefully transfer 200 µL of the supernatant to a new 96-well deep-well plate without disturbing the undigested pellet.

7. Add 200 µL MSL Buffer. Vortex or pipet up and down to mix thoroughly.

8. Incubate at 70°C for 10 minutes.

9. Place the plate on the bench for 5 minutes to bring the plate to room temperature.

10. Add 290 µL 100% ethanol and 10 µL Mag-Bind® Particles SC. Vortex or pipet up and down 20-30 times to mix thoroughly.

11. Let sit at room temperature for 5 minutes.

12. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit for 10-15 minutes.

13. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

14. Remove the plate from the magnetic separation device.

15. Add 400 µL MP Buffer.

    Note: MP Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

16. Resuspend the Mag-Bind® Particles SC by vortexing or pipetting up and down 20-30 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles SC.

17. Let sit for 3 minutes at room temperature. Vortex or pipet up and down a few times during incubation.

18. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

19. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

20. Remove the plate from the magnetic separation device.

21. Add 400 µL SPM Wash Buffer.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

22. Resuspend the Mag-Bind® Particles SC by vortexing or pipetting up and down 20-30 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles SC.

23. Let sit for 3 minutes at room temperature. Vortex or pipet up and down a few times during incubation.

24. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

25. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

26. Repeat Steps 18-23 for a second SPM Wash Buffer wash step.

27. Leave the plate on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles SC. Remove any residual liquid with a pipettor.

28. Remove the plate from the magnetic separation device.

29. Add 50-200 µL Elution Buffer preheated to 70°C.

30. Resuspend the Mag-Bind® Particles SC by vortexing for 3 minutes or pipetting up and down 50 times.

31. Let sit at room temperature for 5-10 minutes.

32. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

33. Transfer the cleared supernatant containing purified DNA to a 96-well microplate.

34. Store DNA at -20°C.

Mag-Bind® Tissue DNA 96 Kit Protocol - Cultured Cells

This protocol is designed for rapid isolation of up to 25 µg genomic DNA from up to 5 x 10^6 cultured cells.

Materials and Equipment to be Supplied by User:

- Magnetic separation device for 96-well plates (Cat# MSD-01B or MSD-01)
- 96-well deep-well plates compatible with the magnetic separation device
- 96-well microplates
- Centrifuge with swing-bucket rotor capable of 4000 x *g*
- Centrifuge adaptor for 96-well plates
- Water bath, incubator, or heat block capable of 70°C
- Shaking water bath capable of 55°C
- Vortexer
- 100% ethanol
- Sealing film
- Multi-channel reservoir
- Cold PBS (4°C)

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4.
- Preheat Elution Buffer to 70°C.
- Set shaking water bath to 55°C.

1. Prepare the cell suspension.

   1a. Frozen cell samples should be thawed before starting this protocol. Pellet cells by centrifugation. Wash the cells with cold PBS (4°C) and resuspend cells in 180 µL cold PBS. Proceed with Step 2 of this protocol.

   1b. For cells grown in suspension, pellet 5 x 10^6 cells at 1,200 x *g* in a centrifuge tube. Discard the supernatant, wash the cells once with cold PBS (4°C), and resuspend cells in 180 µL cold PBS. Proceed with Step 2 of this protocol.

   1c. For cells grown in a monolayer, harvest the cells by either using a trypsin treatment or cell scraper. Wash cells twice in cold PBS (4°C) and resuspend the cells with 180 µL cold PBS. Proceed with Step 2 of this protocol.

2. Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.

   Note: Seal the plate before vortexing to prevent cross-contamination or loss of sample.

3. Incubate at 55°C in a shaking water bath for 10 minutes.

   Note: If a shaking water bath is not available, vortex the sample every 2-3 minutes.

4. Transfer the samples into a 96-well deep-well plate.

5. Add 200 µL MSL Buffer. Vortex to mix thoroughly.

6. Incubate at 70°C for 10 minutes.

7. Place the plate on the bench for 5 minutes to bring to room temperature.

8. Add 260 µL of 100% ethanol, 10 µL Mag-Bind® Particles SC, and 10 µL LPA Buffer. Mix thoroughly by vortexing or pipetting.

9. Let sit at room temperature for 5 minutes.

10. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit for 10-15 minutes.

11. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

12. Remove the plate from the magnetic separation device.

13. Add 400 µL MP Buffer.

    Note: MP Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

14. Resuspend the Mag-Bind® Particles SC by vortexing or pipetting up and down 20-30 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles SC.

15. Let sit for 3 minutes at room temperature. Vortex or pipet up and down a few times during incubation.

16. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

17. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

18. Remove the plate from the magnetic separation device.

19. Add 400 µL SPM Wash Buffer.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

20. Resuspend the Mag-Bind® Particles SC by vortexing or pipetting up and down 20-30 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles SC.

21. Let sit for 3 minutes at room temperature. Vortex or pipet up and down a few times during incubation.

22. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

23. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

24. Repeat Steps 18-23 for a second SPM Wash Buffer wash step.

25. Leave the plate on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles SC. Remove any residual liquid with a pipettor.

26. Remove the plate from the magnetic separation device.

27. Add 50-200 µL Elution Buffer preheated to 70°C.

28. Resuspend the Mag-Bind® Particles SC by vortexing for 3 minutes or pipetting up and down 50 times.

29. Let sit at room temperature for 5-10 minutes.

30. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

31. Transfer the cleared supernatant containing purified DNA to a 96-well microplate.

32. Store DNA at -20°C.

Mag-Bind® Tissue DNA 96 Kit Protocol - Mouse Tail Snips

Materials and Equipment to be Supplied by User:

- Magnetic separation device for 96-well plates (Cat# MSD-01B or MSD-01)
- 96-well deep-well plates compatible with the magnetic separation device
- 96-well microplates
- Centrifuge with swing-bucket rotor capable of 4000 x *g*
- Centrifuge adaptor for 96-well plates
- Water bath, incubator, or heat block capable of 70°C
- Shaking water bath capable of 55°C
- Vortexer
- 100% ethanol
- Sealing film
- Multi-channel reservoir

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4.
- Preheat Elution Buffer to 70°C.
- Set water bath to 55°C.

1. Snip a 2-5 mm piece of mouse tail, cut into several pieces, and transfer the pieces to a 96-well deep-well plate.

   Note: Follow all regulations regarding the safe and humane treatment of animals. Mice should not be older than 6 weeks since lysis will be more difficult in older animals resulting in suboptimal DNA yields. If possible, obtain tail biopsies at 2-4 weeks and freeze samples at -70°C until DNA is extracted.

2. Add 250 µL TL Buffer.

3. Add 20 µL of Proteinase K Solution. Vortex to mix thoroughly.

   Note: Seal the plate before vortexing to prevent cross-contamination or loss of sample.

4. Incubate the plate at 55°C in a shaking water bath for 1-4 hours or until lysis is complete.

   Note: If a shaking water bath is not available, vortex the samples vigorously every 20-30 minutes. Incomplete lysis may significantly reduce DNA yields. Incubation time for complete tail lysis is dependent on length of tail snip and age of animal, e.g. a 5 mm tail piece from a 2 week old mouse typically will lyse in 2 hours. For older animals, an overnight incubation may improve yields. Note that bone and hair will not lyse.

5. Centrifuge at maximum speed for 5 minutes to pellet undigested tissue debris and hair.

6. Carefully transfer 200 µL of the supernatant to a new 96-well deep-well plate without disturbing the undigested pellet.

OPTIONAL: Mouse tail tissue contains RNA that can purify with the DNA. This will not interfere with PCR reactions, but other enzymatic reactions may be affected. To remove RNA, add 5 µL RNase A and let sit at room temperature for 2 minutes.

7. Add 200 µL MSL Buffer. Vortex to mix thoroughly.

8. Incubate at 70°C for 10 minutes.

9. Place the plate on the bench for 5 minutes to bring to room temperature.

10. Add 260 µL of 100% ethanol, 10 µL Mag-Bind® Particles SC, and 10 µL LPA Buffer. Mix thoroughly by vortexing or pipetting.

11. Let sit at room temperature for 5 minutes.

12. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit for 10-15 minutes.

13. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

14. Remove the plate from the magnetic separation device.

15. Add 400 µL MP Buffer.

    Note: MP Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

16. Resuspend the Mag-Bind® Particles SC by vortexing or pipetting up and down 20-30 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles SC.

17. Let sit for 3 minutes at room temperature. Vortex or pipet up and down a few times during incubation.

18. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

19. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

20. Remove the plate from the magnetic separation device.

21. Add 400 µL SPM Wash Buffer.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

22. Resuspend the Mag-Bind® Particles SC by vortexing or pipetting up and down 20-30 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles SC.

23. Let sit for 3 minutes at room temperature. Vortex or pipet up and down a few times during incubation.

24. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

25. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

26. Repeat Steps 20-25 for a second SPM Wash Buffer wash step.

27. Leave the plate on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles SC. Remove any residual liquid with a pipettor.

28. Remove the plate from the magnetic separation device.

29. Add 50-200 µL Elution Buffer preheated to 70°C.

30. Resuspend the Mag-Bind® Particles SC by vortexing for 3 minutes or pipetting up and down 50 times.

31. Let sit at room temperature for 5-10 minutes.

32. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

33. Transfer the cleared supernatant containing purified DNA to a 96-well microplate.

34. Store DNA at -20°C.

Mag-Bind® Tissue DNA 96 Kit Protocol - Buccal Swabs

Materials and Equipment to be Supplied by User:

- Magnetic separation device for 96-well plates (Cat# MSD-01B or MSD-01)
- 96-well deep-well plates compatible with the magnetic separation device
- 96-well microplates
- Centrifuge with swing-bucket rotor capable of 4000 x *g*
- Centrifuge adaptor for 96-well plates
- Water bath, incubator, or heat block capable of 70°C
- Shaking water bath capable of 55°C
- Vortexer
- 100% ethanol
- Sealing film
- Multi-channel reservoir

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4.
- Preheat Elution Buffer to 70°C.
- Set shaking water bath to 55°C.

1. Cut off the buccal brush or swab head and place each swab into a well of a 96-well deep-well plate.

2. Add 400 µL TL Buffer.

3. Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.

   Note: Seal the plate before vortexing to prevent cross-contamination or loss of sample.

4. Incubate at 55°C in a shaking water bath for 45 minutes.

   Note: If a shaking water bath is not available, vortex the plate every 5-10 minutes.

5. Centrifuge at 3000 x *g* for 10 minutes.

6. Transfer 200 µL lysate into a new 96-well deep-well plate. Do not transfer the swabs to the new plate.

7. Add 200 µL MSL Buffer. Vortex to mix thoroughly.

8. Incubate at 70°C for 10 minutes.

9. Place the plate on the bench for 5 minutes to bring to room temperature.

10. Add 260 µL of 100% ethanol, 10 µL Mag-Bind® Particles SC, and 10 µL LPA Buffer. Mix thoroughly by vortexing or pipetting.

11. Let sit at room temperature for 5 minutes.

12. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit for 10-15 minutes.

13. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

14. Remove the plate from the magnetic separation device.

15. Add 400 µL MP Buffer.

    Note: MP Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

16. Resuspend the Mag-Bind® Particles SC by vortexing or pipetting up and down 20-30 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles SC.

17. Let sit for 3 minutes at room temperature. Vortex or pipet up and down a few times during incubation.

18. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

19. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

20. Remove the plate from the magnetic separation device.

21. Add 400 µL SPM Wash Buffer.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

22. Resuspend the Mag-Bind® Particles SC by vortexing or pipetting up and down 20-30 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles SC.

23. Let sit for 3 minutes at room temperature. Vortex or pipet up and down a few times during incubation.

24. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

25. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles SC.

26. Repeat Steps 20-25 for a second SPM Wash Buffer wash step.

27. Leave the plate on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles SC. Remove any residual liquid with a pipettor.

28. Remove the plate from the magnetic separation device.

29. Add 50-200 µL Elution Buffer preheated to 70°C.

30. Resuspend the Mag-Bind® Particles SC by vortexing for 3 minutes or pipetting up and down 50 times.

31. Let sit at room temperature for 5-10 minutes.

32. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles SC. Let sit at room temperature until the Mag-Bind® Particles SC are completely cleared from solution.

33. Transfer the cleared supernatant containing purified DNA to a 96-well microplate.

34. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Low DNA yields	Incomplete resuspension of Mag-Bind® Particles SC	Resuspend the Mag-Bind® Particles SC by vortexing before use.
	Inefficient cell lysis due to inefficient mix of MSL Buffer and sample	Make sure the sample is thoroughly mixed with MSL Buffer.
	SPM Wash Buffer were not prepared correctly.	Prepare the SPM Wash Buffer by adding ethanol according to the instructions
	Loss of magnetic beads during operation	Be careful not to disturb the Mag-Bind® Particles SC during aspiration steps.
	Inefficient cell lysis due to decrease of activity of Proteinase K	Add more Proteinase K Solution.
Problem	Cause	Solution
No DNA eluted	SPM Wash Buffer was not diluted with 100% ethanol	Prepare SPM Wash Buffer as instructed on the Page 4.
Problem	Cause	Solution
Problem with downstream applications	Insufficient DNA was used	• Use more stating material. • Quantify the purified DNA accurately and use sufficient DNA.
	Excess DNA was used	Make sure to use correct amount DNA.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Magnetic separation device for 1.5 mL microcentrifuge tubes	MSD-02
Magnetic separation device for 96-well plates	MSD-01/MSD-01B
Multi-channel reservoir	AC1331-01
Sealing film	AC1200
96-well microplates	EZ9603
SPM Wash Buffer, 40 mL	PS014
Elution Buffer, 100 mL	PDR048
RNase A, 3 mL	RNA-03
Proteinase K Solution, 10 mL	AC116