Auto-Mag® Blood & Tissue DNA Isolation Kits

Cat # AMD-D006 Version 2.0

Protocol for Genomic DNA from Mouse Tail – 96 well format

Materials and Equipment to be supplied by User:

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) from each product supplier.

- 96 well round-bottom deep well plates with a capacity of 1 ml per well.
- Magnetic separation device compatible with 96-well plate
- Centrifuge with swing bucket rotor capable of 4,000 x g
- Shaking water bath
- Vortexer
- 100% Ethanol
- Sealing film for storage
- Optional phosphate-buffered saline (PBS) or nuclease-free water may be required
- Optional RNase A (10 mg/mL)

Before Starting

- Equilibrate samples to room temperature.
- Prepare MPW and TDW buffers according to the instructions of "Preparation of Reagents" and keep them at room temperature.
- Preset water bath, incubator or heating blocks to 65°C and 55°C.
- Preheat Elution Buffer to 65°C.
- Suspension Auto-Mag® Particle D-1 by vortex
- TDB Buffer may show precipitates during storage. If precipitates are present, heat bottle to 37°C to dissolve the precipitates before use.

Procedure

1. Take 2-5mm piece of mouse tail, mince into several piece and add 250µl TSL Buffer.

Note: Cutting/mincing the tissue into smaller pieces can speed up the lysis process. Optional: To improve lysis and reduce incubation time, pulverize sample to a fine powder in liquid nitrogen.

2. Add 20µl Proteinase K Solution and pipette mix 20 times or vortex at maximum speed for 20 seconds. Incubate the sample plate at 55°C in a shaking water bath for overnight.

Note: If a shaking water bath is not available, vortex the sample plate every 20-30 minutes. Lysis time depends on the length of the tail snip and age of the mice. Biopsies should be from 2-4-week-old mice. For older mice, overnight incubation may improve yields.

3. Centrifuge the sample at maximum speed for 5 minutes to pellet the undigested materials. Transfer the clear lysate on top to a new processing plate with a capacity of 1ml per well.

4. Optional: RNA in the mouse tail will be co-purified. If the RNA will interfere with your down steam application, remove the RNA by adding 5µl RNase A and pipette mix 20 times or vortex at maximum speed for 20 seconds.

5. Add 200µl TDB Buffer to the sample and pipette mix 20 times or vortex at maximum speed for 20 seconds. Incubate the sample plate at 65°C for 10 minutes.

6. Bring the sample plate to room temperature. Add 300µl 100 % Ethanol and 10µl Auto-Mag® Particle D-1 to the sample, and pipette mix 20 times or vortex at maximum speed for 20 seconds. Incubate the sample plate at room temperature for 5 minutes.

Note: Thoroughly resuspend the Auto-Mag® Particle D-1 before use.

7. Place the sample plate on the 96-well magnetic separation device and allow the magnetic beads to settle on the magnet for 5 minutes or until the supernatant turns completely clear. Remove and discard the cleared supernatant. Do not disturb the magnetic beads.

8. Remove the sample plate from the 96-well magnetic separation device. Add 500µl MPW Buffer to the sample and resuspend the magnetic beads by pipette mix 20 times or vortex at maximum speed for 20 seconds.

Note: MPW Buffer must be diluted with ethanol prior to use. Complete resuspension of the magnetic beads is crucial for obtaining good purity DNA.

9. Place the sample plate back on the 96-well magnetic separation device and allow the magnetic beads to settle on the magnet for 5 minutes or until the supernatant turns completely clear. Remove and discard the cleared supernatant. Do not disturb the magnetic beads.

10. Remove the sample plate from the 96-well magnetic separation device. Add 500µl TDW Buffer to the sample and resuspend the magnetic beads by pipette mix 20 times or vortex at maximum speed for 20 seconds.

Note: TDW Buffer must be diluted with ethanol prior to use. Complete resuspension of the magnetic beads is crucial for obtaining good purity DNA.

11. Place the sample plate back on the 96-well magnetic separation device again and allow the magnetic beads to settle on the magnet for 5 minutes or until the supernatant turns completely clear. Remove and discard the cleared supernatant. Do not disturb the magnetic beads.

12. Repeat Steps 10 to 11 for a second TDW wash.

13. Keep the sample plate on the magnetic separation device and air dry the magnetic beads by incubating the sample plate at room temperature for 5 minutes.

Note: It is critical to completely remove all liquid from each well.

14. Remove the sample plate from the magnetic separation device. Add 50-100 µl 65°C heated Elution Buffer to the sample and resuspend the magnetic beads by pipette mix 20 times or vortex at maximum speed for 1 minute. Incubate the sample plate at room temperature for 10 minutes.

Note: Incubate at 65°C may improve yield. Complete resuspension of the magnetic beads is crucial for obtaining good purity DNA.

15. Place the sample plate back on the 96-well magnetic separation device and wait for 5 minutes or until the magnetic beads clear from Elution Buffer.

16 Transfer the eluate (cleared supernatant) to an appropriate storage vessel and keep at -20°C for long term storage, or for subsequent applications.