Auto-Mag® Blood & Tissue DNA Isolation Kits

Cat # AMD-D006 Version 2.0

- ❖ Genomic DNA isolation from 50-250 µl of blood, lysate of tissues, mouse tails, cultured cells, or buccal swabs. etc.

Highlights

- Isolate high-quality total DNA from blood and tissue samples
- No organic extraction or alcohol precipitation
- Complete removal of contaminants and inhibitors
- Adaptable to various automated workstations

Protocol Contents

Disclaimers and Safety Information

This kit is designed for research purposes only. The all biological samples are considered potentially infectious. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs).

Trademarks

The trademarks mentioned herein are the property of AMD Biotech Inc. or their respective owners.

Product Introduction

Auto-Mag® Blood & Tissue DNA Isolation Kit is a high-quality genomic purification kit for a variety of sample sources including: 20-250 µl fresh or frozen whole blood, buffy coat containing anticoagulants such as Citrate, EDTA and Heparin, saliva samples, fresh or frozen animal tissues and cells. Up to 96 samples of tissues can be processed in less than an hour. The kit utilizes our proprietary magnetic beads chemistry and requires no phenol or chloroform extraction or alcohol precipitation and is suited for high throughput automation. The purified high-quality genomic DNA is suitable for direct use in most downstream applications such as amplification and enzymatic reactions.

Application

Blood and tissue gDNA extraction for:

- PCR, Real-time PCR
- Southern Blotting
- Cloning, Genotyping
- Sequencing

Kit Contents

Product Number	AMD-D006-10	AMD-D006-96	AMD-D006-96X4	AMD-D006-96X20
Preparations	10	96	384	1920
Auto-Mag® D-1	0.12 ml	1 ml	4 ml	20 ml
TSL Buffer	5 ml	40 ml	160 ml	800 ml
TDB Buffer	4 ml	30 ml	125 ml	625 ml
MPW Buffer*	3.2 ml	24 ml	96 ml	480 ml
TDW Buffer *	4 ml	32 ml	128 ml	280 ml x 2
Elution Buffer	1.5 ml	15 ml	60 ml	350 ml
Proteinase K Solution	0.21 ml	2.1 ml	8.5 ml	40 ml

* Ethanol must be added prior to use. See Preparation of Reagents

Storage and Stability

Auto-Mag® Blood & Tissue Isolation Kit is shipped at room temperature and is guaranteed for at least 12 months from the date of purchase when the components of kit are stored as follows: Auto-Mag® D-1 and Proteinase K Solution can be store at 2-8°C. Store all other components at room temperature (22-25°C). Check buffers for precipitates before use. Re-dissolve any precipitates by warming to 37˚C.

Preparation of Reagents

Prepare the following components for each kit before use:

- Dilute MPW and TDW Buffer with 100% Ethanol as follows and store at room temperature

	100% Ethanol to be added	
Kit	MPW	TDW
AMD-D006-10	4 ml	10 ml
AMD-D006-96	30 ml	80 ml
AMD-D006-96X4	120 ml	320 ml
AMD-D006-96x20	600 ml	700 ml x 2

Amounts of starting material

Use the amounts of starting material indicated in Table.

Sample	Amount
Blood	50-250 µl
Saliva	50-250 µl
Most tissue samples	10 mg
Spleen	5-6 mg

Protocol for Genomic DNA from 100-250µl Blood – 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.

- 1.2 ml deep-well plate
- 96-well magnetic separation device for 1.2 ml deep well plate
- Sealing film for storage
- 100% Ethanol
- Optional phosphate-buffered saline (PBS) or nuclease-free water may be required
- Optional RNase A (10 mg/mL)
- Optional: 70% Ethanol, for additional DNA wash.

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® 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. Add 100-250µl blood sample to a well of 1.2 ml deep-well plate. If sample volume is less than 250µl, bring sample volume up to 250µl with the PBS or the Elution Buffer.

2. Add 20µl Proteinase K Solution to the sample plate and pipette mix 20 times or vortex at maximum speed for 20 seconds.

3. Optional: Add 5µl RNase A to the sample and pipette mix 20 times or vortex at maximum speed for 20 seconds.

4. Add 300µl TDB Buffer to the sample and pipette mix 20 times or vortex at maximum speed for 20 seconds.

5. Incubate the sample plate at 65°C for 10 minutes. Mix the samples once during the incubation.

6. Add 400µl 100 % Ethanol and 10µl Auto-Mag® 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® 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 50-100µl 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 the sample plate 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.

Protocol for Genomic DNA from Saliva – 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.

- 1.2 ml deep-well plate
- 96-well magnetic separation device for 1.2 ml deep well plate
- Sealing film for storage
- 100% Ethanol
- Optional phosphate-buffered saline (PBS) or nuclease-free water may be required
- Optional RNase A (10 mg/mL)
- Optional: 70% Ethanol, for additional DNA wash.

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® 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. Add 100-250µl Saliva sample to a well of 1.2ml deep-well plate. If sample volume is less than 250µl, bring sample volume up to 250µl with the PBS or the Elution Buffer.

2. Add 10µl Proteinase K Solution to the sample plate and pipette mix 20 times or vortex at maximum speed for 20 seconds.

3. Optional: Add 5µl RNase A to the sample and pipette mix 20 times or vortex at maximum speed for 20 seconds.

4. Add 300µl TDB Buffer to the sample and pipette mix 20 times or vortex at maximum speed for 20 seconds.

5. Incubate sample plate at room temperature for 10 minutes. Mix the sample once during the incubation.

6. Add 300µl 100 % Ethanol and 10µl Auto-Mag® 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 10 minutes.

Note: Thoroughly resuspend the Auto-Mag® 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 50-100µl 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 the sample plate 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.

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Protocol for Genomic DNA from Tissues - 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)
- Optional: 70% Ethanol, for additional DNA wash.

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® 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. Place up to 10mg tissue into a well of a 96 deep-well plate. 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.

For spleen tissue, use 5-6mg. This will reduce the thickness of the gDNA extracted solution and allow a more efficient wash and ultimately a better-quality extracted DNA.

2. Add 20µl Proteinase K Solution to each sample well. Seal the plate and vortex at maximum speed for 20 seconds. Incubate the sample plate at 55°C in a shaking water bath overnight. Overnight lysis is recommended for optimal yield

Alternatively, lysis can be performed in 2-4 hours depending on the amount and tissue type. If a shaking water bath is not available, vortex the plate every 20-30 minutes.

3. Quickly spin plate for 20 seconds to collect liquid.

Note: For tissues samples containing material that cannot be digested during the lysis step, centrifuge the plate at maximum speed for 5 minutes to pellet the undigested materials. Transfer the clear lysate on top to a new processing plate.

4. Optional: Add 5µl RNase A to the sample 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 room temperature for 10 minutes.

6. Add 300µl 100 % Ethanol and 10 µl Auto-Mag® 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® 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 50-100µl 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 the sample plate 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.

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)
- Optional: 70% Ethanol, for additional DNA wash.

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® 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 1 ml 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 room temperature for 10 minutes.

6. Add 300µl 100 % Ethanol and 10µl Auto-Mag® 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® 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.

Protocol for Genomic DNA from Culture cells – 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)
- Optional: 70% Ethanol, for additional DNA wash.
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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® 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. Prepare the cultured cell suspension according to your starting sample method:

a. 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 200µl TSL. Proceed with Step 2 of this protocol.

b. 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 in 200µl TSL. Proceed with Step 2 of this protocol.

c. 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 in 200µl TSL. Proceed with Step 2 of this protocol.

2. Add 20µl Proteinase K Solution to each sample and pipette mix 20 times or vortex at maximum speed for 20 seconds. Incubate at 55°C in a water bath for 10 minutes.

3. Optional: 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.

4. Transfer the samples to a new 96-well deep well plate.

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 room temperature for 10 minutes.

6. Bring sample plate to room temperature. Add 300µl 100 % Ethanol and 10µl Auto-Mag® 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® 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 50-100µl 65°C heated Elution Buffer to the sample and resuspend the magnetic bead 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 the sample plate 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.

Protocol for Genomic DNA from Buccal Swabs – 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)
- Optional: 70% Ethanol, for additional DNA wash.

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® 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. Cut off the buccal brush or swab head and place into a well of a 96 well deep well plate:

2. Add 400µl TSL Buffer, and 20µl Proteinase K Solution to each sample and pipette mix 20 times or vortex at maximum speed for 20 seconds. Incubate the sample plate at 55°C in a water bath for 45 minutes.

3. Optional: 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.

4. Centrifuge the sample plate at 3,000 x g for 10 minutes. Transfer 200µl lysate to a new 96-well deep well plate.

Note: Do not transfer the swabs or other debris.

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 room temperature for 10 minutes.

6. Add 300µl 100 % Ethanol and 10µl Auto-Mag® D-1 to the sample and by 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® 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 50-100µl 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 the sample plate 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.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via: Phone: 1-404-259-6276 (in US), Email: support@amdbiotech.com

Symptoms	Possible Causes	Comments
Low DNA yields	Incomplete resuspension of Auto-Mag® D-1 beads	Resuspend Auto-Mag® D-1 beads by vortexing vigorously before use.
	Loss of Auto-Mag® D-1 beads during operation	Avoid disturbing the Auto-Mag® D-1 beads during aspiration of supernatant.
	DNA remains bound to the Auto-Mag® D-1 beads	Increase elution volume and incubate for 15 minutes. Pipet mix 50 to 100 times
	Ethanol is not added into HSW Buffer	Add absolute 100% Ethanol to HSW Buffer (see Page 2 for instructions).
Auto-Mag® D-1 beads do not clear from solution	Too short of magnetizing time	Increase collection time on the magnet
Downstream applications are unsuccessful	Insufficient DNA in starting material	Use more starting material
	Ethanol carry-over	Dry the Auto-Mag® D-1 beads completely before elution.