AMD Biotech Inc.
Advanced Molecular Detection

Product Manual

Auto-Mag® Blood & Tissue DNA Isolation Kit

Version 2.1

The magnetic bead-based kit for automated or manual isolation of high-quality genomic DNA from blood, tissue lysates, mouse tails, cultured cells, or buccal swabs, etc.

Catalog Number: D032-00, D032-01, D032-02,

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Disclaimers and Safety Information

This kit is designed for research use only. All biological samples are considered potentially infectious. 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). MSDS can be downloaded from the "Product Documents" tab when viewing the product kit. Download MSDS at www.amdbiotech.com. Information in this document is subject to change without notice.

Product Introduction

Auto-Mag® Blood & Tissue DNA Isolation Kit uses magnetic bead-based workflows and combines multiple sample processing steps together for high quality genomic DNA purification from various sources including fresh or frozen whole blood, buffy coat, saliva, fresh or frozen mammalian tissues and cells lines. Up to 96 samples of tissues can be processed in less than an hour. Purified high-quality genomic DNA is suitable for direct use in most common molecular biology applications: PCR, conventional restriction digestion, cloning, DNA sequencing, and Southern blot analysis.

Auto-Mag® Blood & Tissue DNA Isolation Kit uses a simple 4 step procedure: Lyse-Bind-Wash-Elute. Follow the instructions, the extraction can be performed manually or on automated platforms with equivalent results.

Features:

- Fast and easy processing using a magnetic bead system.
- Isolate high-quality Genomic DNA from various sources samples.
- No phenol or chloroform extraction or alcohol precipitation
- Complete removal of contaminants and inhibitors
- Additional Automation-ready protocols for IsoPure Mini, IsoPure 96, NAP-16 or KingFisher system, etc.

Kit Contents

Product Number	**D032-00**	**D032-01**	**D032-02**
Preparation	5	96	384
Auto-Mag® D-1	0.6 ml	1.2 ml	4.4 ml
TSL Buffer	2 ml	40 ml	160 ml
TDB Buffer	2 ml	40 ml	160 ml
MDW buffer*	3 ml	40 ml	80 ml x2
DNA Elution Buffer	1 ml	20 ml	80 ml
Proteinase K Solution	0.11 ml	2 ml	8 ml
RNase A	0.03 ml	0.5 ml	2 ml
*** Ethanol must be added prior to use. See Preparation of Reagents**			

Storage and Stability

Auto-Mag® Blood & Tissue Isolation Kit is shipped at ambient temperature. All components are stable for 12 months when stored accordingly. Auto-Mag® D-1, Proteinase K Solution and RNase A can be stored at room temperature (15-25°C) for 12 months, to prolong the shelf-life, storage at 2-8°C is recommended. All other components can be stored at room temperature (15-25°C).

During shipment or storage in cool ambient conditions, the precipitates may form in some buffers. Check buffers and re-dissolve any precipitates by warming the buffer at 37˚C. and gently shaking before using.

Preparation of Reagents

1. Dilute MDW buffer with 100% Ethanol as follows and store at room temperature.

Reagents	Kit	100% Ethanol to be Added
MDW buffer	D032-00	2 ml
	D032-01	40 ml
	D032-02	80 ml per bottle
Components are stable for 1 year when stored closed at room temperature		

2. Prepare 70% Ethanol for DNA Wash, and prepare at least 1.6 ml for a prep.

Additional Information

1. Specifications

Features	**Specification**
Isolation Technology	Magnetic Beads
Sample Sources	Blood, saliva, tissue, cells, or buffy coat, etc.
Starting Amount	Up to 200 µl or 30 mg
Typical Yield	Dependent upon sample, (2-30µg)
A260/280	1.8-2.0
Elution Volume	50-100 µl
Processing format	Automated; Manual
Downstream Application	qPCR, PCR, NGS. etc.

2. Amounts of starting material

Sample	**Amount**
Blood	20-300µl
Saliva	50-200µl
Most tissue samples	10 mg
Spleen	5-6 mg

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Auto-Mag® Blood & Tissue DNA Isolation Protocols

Materials and Equipment to Be Supplied by User

- Single-tube format: Nuclease-free 1.5-2.0 ml microcentrifuge tube, and Magnetic Rack Separator for microcentrifuge tube.
- 96-well format: 1.2 ml deep-well plate. Compatible magnetic separation device for 96-well plate
- Sealing film for 96 well plate
- Benchtop vortex mixer
- Water bath, incubator, or heat block capable of 55°C
- 100% Ethanol
- 70% Ethanol
- Optional: phosphate-buffered saline (PBS) or nuclease-free water may be required

Before Starting

- Equilibrate samples to room temperature.
- Ensure MDW buffer is prepared according to the instructions of Preparation Reagents on page 3.
- Prepare 70% Ethanol for DNA wash steps.
- Preset water bath, incubator, or heating blocks to 55°C.
- TDB Buffer may show precipitates during storage. If precipitates are present, heat bottle to 37°C before use.
- Complete resuspension of the Auto-Mag® D-1 by vortex.

Protocol for 20-100µl Whole Blood Samples (single tube or 96 well format)

1. Transfer 20-100µl blood sample to a 1.5-2.0 ml microcentrifuge tube, or each well of 1.2 ml deep-well plate. Bring sample volume up to 200µl with the PBS or the Elution Buffer.
2. Add 20µl Proteinase K Solution, 5µl RNase A to the sample and pipette mix 20 times or vortex for 20 seconds.

   Note: if using 96 well plate, Seal the 96-well plate with sealing film before vortex mix.
3. Add 300µl TDB Buffer to the sample and pipette mix 20 times or vortex for 20 seconds.
4. Incubate the sample at 55°C for 30 minutes. Mix the samples once or twice during the incubation.

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

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

6. Place the sample tube or plate on a compatible magnetic separation device for 5 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

7. Remove the sample from the magnet. Add 800µl MDW buffer and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

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

8. Place the sample on the compatible magnet for 5 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

9. Remove the sample from the magnet. Add 800µl 70% Ethanol and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

10. Place the sample on the compatible magnet for 2 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

11. Repeat Steps 10 -11 for a second 70% Ethanol wash.

12. Keep the sample tube or plate on the compatible magnet, and air dry the magnetic beads at room temperature for 5 minutes. Remove any residue liquid with a pipettor.

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

13. Add 50~100 µl Elution Buffer to the sample and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

14. Incubate the sample at 55°C for 5 minutes.

15. Place the sample tube or plate back on the compatible magnet for 5 minutes or until the Auto-Mag® D-1 beads are completely cleared 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 100-300µl whole Blood Samples (single tube or 96 well format)

1. Transfer 100-300µl blood sample to a 1.5-2.0 ml microcentrifuge tube, or each well of 2 ml deep-well plate. If sample volume is less than 300µl, bring sample volume up to 300µl with the PBS or the Elution Buffer.

2. Add 20µl Proteinase K Solution, 5µl RNase A to the sample and pipette mix 20 times or vortex for 20 seconds.

   Note: if using 96 well plate, Seal the 96-well plate with sealing film before vortex mix.

3. Add 400µl TDB Buffer to the sample and pipette mix 20 times or vortex for 20 seconds.

4. Incubate the sample at 55°C for 30 minutes. Mix the samples once or twice during the incubation.

5. Bring samples to room temperature. Add 400µl 100 % Ethanol and 10µl Auto-Mag® D-1 to the sample, and pipette mix 20 times or vortex for 20 seconds. Incubate at room temperature for 5 minutes.

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

6. Place the sample tube or plate on a compatible magnetic separation device for 5 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

7. Remove the sample from the magnet. Add 800µl MDW buffer and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

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

8. Place the sample on the compatible magnet for 2 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

9. Remove the sample from the magnet. Add 800µl 70% Ethanol and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

10. Place the sample on the compatible magnet for 2 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

11. Repeat Steps 10 -11 for a second 70% Ethanol wash.

12. Keep the sample tube or plate on the compatible magnet, and air dry the magnetic beads at room temperature for 5 minutes. Remove any residue liquid with a pipettor.

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

13. Add 100~150 µl Elution Buffer to the sample and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

14. Incubate the sample at 55°C for 5 minutes.

15. Place the sample tube or plate back on the compatible magnet for 5 minutes or until the Auto-Mag® D-1 beads are completely cleared 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 Animal Tissue Samples (single tube or 96 well format)

Additional Materials and Equipment to Be Supplied by User

- Centrifuge capable of 3000–4000 × g with swinging-bucket rotor for 96-well deep well plates
- Shaking water bath

1. Place up to 10mg tissue into a 1.5-2.0 ml microcentrifuge tube, or each well of 1.2 ml deep-well plate. Add 200µl TSL Buffer. Ensure that the tissue is completely immersed in the Lysis 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 washing and ultimately better-quality extracted DNA.

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

   Note: if using 96 well plate, Seal the 96-well plate with sealing film before vortex mix.

   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.

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

3. Optional: Add 5µl RNase A to each sample and pipette mix 20 times or vortex for 20 seconds. Incubate at room temperature for 5 minutes.

4. Add 300µl TDB Buffer to the sample and pipette mix 20 times or vortex for 20 seconds. Incubate the sample plate at room temperature for 10 minutes. Mix the samples once during the incubation.

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

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

6. Place the sample tube or plate on a compatible magnetic separation device for 5 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

7. Remove the sample from the magnet. Add 800µl MDW buffer and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

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

8. Place the sample on the compatible magnet for 2 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

9. Remove the sample from the magnet. Add 800µl 70% Ethanol and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

10. Place the sample on the compatible magnet for 2 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

11. Repeat Steps 10 -11 for a second 70% Ethanol wash.

12. Keep the sample tube or plate on the compatible magnet, and air dry the magnetic beads at room temperature for 5 minutes. Remove any residue liquid with a pipettor.

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

13. Add 50~100 µl Elution Buffer to the sample and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

14. Incubate the sample at 55°C for 5 minutes.

15. Place the sample tube or plate back on the compatible magnet for 5 minutes or until the Auto-Mag® D-1 beads are completely cleared 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 Mouse Tail Sample (single tube or 96 well format)

1. Take 5-8 mm piece of mouse tail, mince into several pieces, and put into a 1.5-2.0 ml microcentrifuge tube, or each well of 1.2 ml deep-well plate. Add 300µl TSL Buffer. Ensure that the tissues are completely immersed in the 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 for 20 seconds. Incubate the sample plate at 55°C in a shaking water bath overnight.

   Note: if using 96 well plate, Seal the 96-well plate with sealing film before vortex mix.

   If a shaking water bath is not available, vortex the sample 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 ≥ 16,000g at room temperature for 5 minutes to pellet the undigested materials. Transfer 200µl the clear lysate on top to a new tube or plate with a capacity of 1.2 ml per well.

   Note: 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 for 20 seconds.

Incubate at room temperature for 5 minutes.

4. Add 300µl TDB Buffer to the sample and pipette mix 20 times or vortex for 20 seconds. Incubate the sample at room temperature for 10 minutes. Mix the samples once during the incubation.

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

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

6. Place the sample tube or plate on a compatible magnetic separation device for 5 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

7. Remove the sample from the magnet. Add 800µl MDW buffer and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

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

8. Place the sample on the compatible magnet for 5 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

9. Remove the sample from the magnet. Add 800µl 70% Ethanol and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

10. Place the sample on the compatible magnet for 2 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

11. Repeat Steps 10 -11 for a second 70% Ethanol wash.

12. Keep the sample tube or plate on the compatible magnet, and air dry the magnetic beads at room temperature for 5 minutes. Remove any residue liquid with a pipettor.

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

13. Add 50~100 µl Elution Buffer to the sample and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

14. Incubate the sample at 55°C for 5 minutes.

15. Place the sample tube or plate back on the compatible magnet for 5 minutes or until the Auto-Mag® D-1 beads are completely cleared 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 cultured cell (single tube or 96 well format)

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 at 1,200 x g for 5 minutes. Wash the cells with 500µl cold PBS (4°C) once and resuspend cells in 200µl TSL buffer. Proceed with Step 2 of this protocol.

   b. For cells grown in suspension, pellet $5x10^6$ cells by centrifuge at 1,200 x g for 5 minutes. Discard the supernatant, wash the cells once with 500µl cold PBS (4°C) once and resuspend cells in 200µl TSL buffer. 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 with 500µl cold PBS (4°C) twice and resuspend the cells with 200µl TSL buffer. Proceed with Step 2 of this protocol.

2. Transfer the 200µl sample to a 1.5-2.0 ml microcentrifuge tube, or each well of 1.2 ml deep-well plate. Add 20µl Proteinase K Solution and pipette mix 20 times or vortex for 20 seconds. Incubate at 55°C in a water bath for 15 minutes.

   Note: if using 96 well plate, Seal the 96-well plate with sealing film before vortex mix.

3. Add 5µl RNase A to the sample and pipette mix 20 times or vortex for 20 seconds. Incubate at room temperature for 5 minutes.

4. Add 300µl TDB Buffer to the sample and pipette mix 20 times or vortex for 20 seconds. Incubate the sample at room temperature for 10 minutes. Mix the samples once during the incubation.

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

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

6. Place the sample tube or plate on a compatible magnetic separation device for 5 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

7. Remove the sample from the magnet. Add 800µl MDW buffer and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

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

8. Place the sample on the compatible magnet for 2 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

9. Remove the sample from the magnet. Add 800µl 70% Ethanol and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

10. Place the sample on the compatible magnet for 2 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

11. Repeat Steps 10 -11 for a second 70% Ethanol wash.

12. Keep the sample tube or plate on the compatible magnet, and air dry the magnetic beads at room temperature for 5 minutes. Remove any residue liquid with a pipettor.

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

13. Add 50~100 µl Elution Buffer to the sample and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

14. Incubate the sample at 55°C for 5 minutes.

15. Place the sample tube or plate back on the compatible magnet for 5 minutes or until the Auto-Mag® D-1 beads are completely cleared 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 20-200µl Saliva Samples (single tube or 96 well format)

1. Transfer 50-200µl saliva sample to a 1.5-2.0 ml microcentrifuge tube, or each well of 1.2 ml deep-well plate. If sample volume is less than 200µl, bring sample volume up to 200µl with the PBS or the Elution Buffer.

2. Add 20µl Proteinase K Solution, 5µl RNase A to the sample and pipette mix 20 times or vortex for 20 seconds.

   Note: if using 96 well plate, Seal the 96-well plate with sealing film before vortex mix.

3. Add 300µl TDB Buffer to the sample and pipette mix 20 times or vortex for 20 seconds.

4. Incubate the sample at 55°C for 30 minutes. Mix the samples once or twice during the incubation.

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

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

6. Place the sample tube or plate on a compatible magnetic separation device for 5 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

7. Remove the sample from the magnet. Add 800µl MDW buffer and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

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

8. Place the sample on the compatible magnet for 2 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

9. Remove the sample from the magnet. Add 800µl 70% Ethanol and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

10. Place the sample on the compatible magnet for 2 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

11. Repeat Steps 10 -11 for a second 70% Ethanol wash.

12. Keep the sample tube or plate on the compatible magnet, and air dry the magnetic beads at room temperature for 5 minutes. Remove any residue liquid with a pipettor.

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

13. Add 50~100 µl Elution Buffer to the sample and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

14. Incubate the sample at 55°C for 5 minutes.

15. Place the sample tube or plate back on the compatible magnet for 5 minutes or until the Auto-Mag® D-1 beads are completely cleared 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 Buccal Swabs Samples (single tube or 96 well format)

1. Cut off the buccal brush or swab head and place into a 1.5-2.0 ml microcentrifuge tube, or each well of 2 ml deep-well plate.:

2. Add 400µl TSL Buffer, and 20µl Proteinase K Solution to each sample and pipette mix 20 times or vortex 20 seconds. Incubate the sample plate at 55°C in a water bath for 30 minutes.

   Note: if using 96 well plate, Seal the 96-well plate with sealing film before vortex mix.

3. Centrifuge the sample at 3,000 x g for 10 minutes. Transfer 200µl lysate to a new 1.5-2.0 ml microcentrifuge tube, or each well of 1.2 ml deep-well plate

   Note: 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 20 seconds. Incubate at room temperature for 5 minutes.

4. Add 300µl TDB Buffer to the sample and pipette mix 20 times or vortex for 20 seconds. Incubate the sample at room temperature for 10 minutes. Mix the samples once during the incubation.

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

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

6. Place the sample tube or plate on a compatible magnetic separation device for 5 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

7. Remove the sample from the magnet. Add 800µl MDW buffer and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

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

8. Place the sample on the compatible magnet for 2 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

9. Remove the sample from the magnet. Add 800µl 70% Ethanol and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

10. Place the sample on the compatible magnet for 2 minutes or until Auto-Mag® D-1 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

11. Repeat Steps 10 -11 for a second 70% Ethanol wash.

12. Keep the sample tube or plate on the compatible magnet, and air dry the magnetic beads at room temperature for 5 minutes. Remove any residue liquid with a pipettor.

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

13. Add 50~100 µl Elution Buffer to the sample and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

14. Incubate the sample at 55°C for 5 minutes.

15. Place the sample tube or plate back on the compatible magnet for 5 minutes or until the Auto-Mag® D-1 beads are completely cleared 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 and Programmed Procedure for Automated Isolation Process

If automating these procedures on a liquid handler or a magnetic processor, please contact AMD Biotech for instrument-specific instructions and additional processing procedures.

Phone: 404-290-5063 (in US), Email: support@amdbiotech.com

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via: Phone: 404-290-5063 (in US), Email: support@amdbiotech.com

Symptoms	Possible Causes	Comments
Low DNA yields	Frozen samples not mixed properly after thawing	Thaw the frozen sample at room temperature and gently mix the sample by inverting.
	Incomplete resuspension of Auto-Mag® D-1 beads	Resuspend Auto-Mag® D-1 beads by vortexing vigorously before use.
	Loss of Auto-Mag® D-1beads during operation	Avoid disturbing the Auto-Mag® beads during aspiration of supernatant.
	DNA remains bound to the Auto-Mag® D-1 beads	Increase elution volume and incubate at 65°C for 10 minutes. Pipet mix 50 times or vortex for 50 seconds
	Ethanol is not added into MDW buffer	Add absolute 100% Ethanol to MDW buffer (see Page 3 for instructions).
Auto-Mag® 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.

Ordering Information

Product Description	Catalog No.	Size
Auto-Mag® Blood/Tisue DNA Isolation Kit	D032-00 D032-01 D032-02 D032-Bulk	5 Preps 96 Preps 384 Preps Request

AMD Biotech is committed to simplifying your research with quality products. and services.

If you are dissatisfied with this product for any reason, please call: 404-290-5063

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