AMD Biotech Inc.
Advanced Molecular Detection

Product Manual

Auto-Mag® FFPE Tissue DNA Isolation Kit

Version 2.1

Xylene-free, Magnetic bead-based reagent for automated or manual isolation of DNA from FFPE tissue samples

Catalog Number: D008-00, D008-01, D008-02,

Contents

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® FFPE Tissue DNA Isolation Kit utilizes the paramagnetic bead-based technology and is specially designed to isolate DNA from formalin-fixed, paraffin-embedded (FFPE) tissue without the use of xylene. The Kit provides a special deparaffinization reagents and compatible extraction buffer for DNA isolation. This combination allows the entire process of isolation to be performed in the same tube, minimizing sample loss and cross-contamination. The purified DNA is of high yield and integrity and is free of inhibitors, ready for use in several downstream applications including qPCR, mutation screening, microarray analysis, sequencing, single nucleotide polymorphism (SNP) and short-tandem repeat (STR) genotyping, etc.

The isolation can be performed in both 96-well plates (manually and automated) and in 1.5-2.0 ml tubes (manually only) with related protocols. Nucleic acid extraction begins with the solubilization of the paraffin from the tissue slices first. An enzymatic lysis step digests the tissue and releases the nucleic acids, followed by decrosslinking at a high temperature. The binding solution is added to immobilize the nucleic acids to the surface of the Auto-Mag® D-3 beads. Contaminants are rinsed away using a simple washing procedure and the nucleic acids are eluted with elution buffer.

Features

- No Xylene – avoid toxic chemicals.
- No centrifuge needed and Automation-friendly.
- Auto-Mag® D-3 beads are compatible with deparaffinization reagent –No need rid of deparaffinization reagent during isolation processing.
- Sample loss and Cross contamination is eliminated as each sample remains in the same tube device during all isolation steps.
- Minimal steps required and convenient processing makes it suitable for all levels of expertise.
- Eluted DNA is suitable for most molecular downstream applications.

Kit Contents

Product Number	D008-00	D008-01	D008-02
Preparation	5	50	200
Auto-Mag® D-3	0.06 ml	0.6 ml	2.2 ml
FDR **(**FFPE Deparaffinization Reagent)	3 ml	28 ml	110 ml
FTL Buffer	1.2 ml	12 ml	48 ml
FDB Buffer	1.8 ml	18 ml	72 ml
FDW Buffer*	5 ml	50 ml	90 ml x2
DNA Elution Buffer	1 ml	10 ml	40 ml
Proteinase K Solution	0.11 ml	1.1 ml	4.2 ml
RNase A	0.03 ml	0.3 ml	1.1 ml
* Ethanol must be added prior to use. See Preparation of Reagents			

Storage and Stability

Auto-Mag® FFPE Tissue DNA Isolation Kit is shipped at ambient temperature. All components are stable for 12 months when stored accordingly. Auto-Mag® D-3, 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 FDW Buffer with 100% Ethanol as follows and store at room temperature.

Reagents	Kit	100% Ethanol to be Added
FDW Buffer	D008-00	5 ml
	D008-01	50 ml
	D008-02	90 ml per bottle
Components are stable for 1 year when stored closed at room temperature		

2. Fresh prepare 80% ethanol for all FFPE DNA wash steps and prepare at least 1.6 ml per test.

Additional Information

1. Specification

Features	**Specification**
Isolation Technology	Magnetic Beads
Sample Sources	formalin-fixed, paraffin-embedded tissue section sample
Starting Amount	< 3 FFPE sections of 10μm thickness each
Typical Yield	Dependent upon sample
A260/280	1.6-1.9
Elution Volume	30-100 μl
Processing format	Auto, Manual
Downstream Application	NGS, qPCR, mutation screening, microarray analysis, sequencing, SNP, and STR genotyping, analysis etc.

2. Important Information about FFPE Samples.

Auto-Mag® FFPE Tissue DNA Isolation Kit utilizes is recommended for the isolation of DNA from formalin-fixed, paraffin-embedded (FFPE) tissue samples. Samples are typically thin sections (approx. 3–20µm thickness) of human or animal origin usually obtained by tissue resection or biopsy. Many factors influence the yield and quality of DNA obtained from FFPE samples. The procedure of tissue sampling, post sampling delay before fixation, fixation time, embedding, and storage conditions have a high impact on DNA quality and yield. The recommended amount of paraffin is up to 10mg when using the standard protocol with 500µl FFPE Deparaffinization Reagent (FDR). DNA isolated from formalin-fixed, paraffin-embedded tissue shows size distribution from 50bp to 5,000bp. Predominantly DNA of approx. 100–300 bases is observed, especially when the sample material is old. However, samples which were subjected to good tissue fixation, embedding, and storage conditions can yield DNA even larger than 5,000 bp. DNA preparation time strongly depends on the sample and the required lysis time. For the best results lysis is performed at 55oC for at least one hour. For some kinds of samples, a longer lysis (e.g., overnight) will even result in remarkably higher DNA yield.

3. Kits include enough reagents for the specified number of preparations. Please be aware that the actual number of preparations may be lower due to pre-aliquoting of reagents, processing partial plates, and automation platform used etc. Additional reagents are available for purchase separately. Please visit the product page at www.amdbiotech.com or contact AMD Biotech for more details and ordering information.

Auto-Mag® FFPE Tissue DNA Isolation Protocols

Materials and Equipment to Be Supplied by User

- Single-tube format: Nuclease-free 1.5-2.0ml microcentrifuge tube, and Magnetic Rack Separator microcentrifuge tube
- 96-well format: 96-well plates with at least a 2 ml capacity and compatible magnetic separation device for 96-well plate
- Sealing film for 96 well plate
- Water bath, incubator, or heat block capable of 55°C and 90°C
- Vortex
- 80% ethanol
- 100% isopropanol

Before Starting

- Please read this booklet in its entirety to become familiar with the isolation procedures.
- Ensure FDW Buffer is prepared according to the instructions of Preparation Reagents on page 3.
- Prepare 80% ethanol for FFPE DNA wash steps. Do not use a previously prepared solution.
- Preset water bath, incubator, or heating blocks to 55°C.
- Preset water bath, incubator, or heating blocks to 90°C.
- FDR and FDB Buffer may show precipitates during storage. If precipitates are present, heat the bottle to 37°C before use.
- Complete resuspension of the Auto-Mag® D-3 beads by vortex

Protocol for Single-tube Format (1.5-2.0 ml Microcentrifuge Tube)

1. Prepare the FFPE sample

   1). Prepare the curls from FFPE tissue blocks: Cut sections from FFPE tissue blocks using a microtome. Collect and transfer 1-3 sections into a 1.5-ml microcentrifuge tube.

   2). Prepare samples from FFPE slides: Scrape the tissue sections in a single direction with a clean razor blade or scalpel, then collect the tissue on the slide into a cohesive mass. Transfer the tissue mass into a 1.5ml tube with the scalpel or a pipette tip.

Note: For FFPE slide, 1 cm^2 FFPE sample weight about ~2mg, do not add over 10mg.

2. Add 500µl FDR to each sample. Ensure that the tissues are completely immersed in the FDR.

3. Incubate the sample tube at 90°C for 5 minutes to solubilize the paraffin and disperse the tissue. Vortex briefly once during incubation.

4. Remove the tubes from the heat source, add 200µl FTL Buffer and 20µl Proteinase K to each sample. Gently flip the sample tube upside down 5 times to disperse the tissue into the FTL buffer.

   Note: When working with multiple samples, FTL buffer, and Proteinase K can be prepared as a mix to save pipetting steps. Prepare only what is needed for each run.

5. Incubate the sample tubes at 55°C for 1 hour. If necessary, extend the incubation time to 4 hours or even overnight to achieve complete lysis, briefly mix by flip tube 2-5 times during incubation.

6. Transfer sample tubes to 90°C, incubate for 1 hour for decrosslinking. Then, remove the sample from the heat source and leave at room temperature for 5 minutes.

   Note: Decrosslinking step is strongly recommended for short time lysis (1–4 h) and may be omitted after overnight Lysis at step 5.

7. Add 5µl RNase A to each sample, and gently mix by flip the sample tube upside down 5 times. incubate the sample tube at room temperature for 10 minutes.

8. Add 300µl FDB buffer, 10µl Auto-Mag® D-3, 300µl 100% isopropanol to each sample, vortex at maximum speed for 20 seconds.

   Note: When working with multiple samples, FDB buffer, and Auto-Mag® D-3, and 100% isopropanol can be prepared as master mix. Mix well and add 610 µl for each sample. Prepare only what is needed for each run.

9. Incubate samples at room temperature for 10 minutes.

   Note: There will be two layers, the top layer is FDR with the paraffin, and the bottom layer is an aqueous phase with DNA and magnetic beads. If the paraffin content is too high, there may be some beads that are in the top layer. If this happens, at step 10, keep the sample tube on the magnet, gently shacking the sample tube several times to clear the beads from the top layer.

10. Place the sample tubes on a compatible magnetic separation device for 5 minutes or until Auto-Mag® D-3 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

11. Remove the sample tube from the magnet. Add 800µl FDW Buffer and resuspend the Auto-Mag® D-3 beads by vortex at maximum speed for 20 seconds or pipette mix 20 times.

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

12. Place the sample tube on the magnet for 2 minutes or until Auto-Mag® D-3 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

13. Repeat Steps 11-12 for a second FDW buffer wash.

14. Remove the sample tube from the magnet. Add 800µl of freshly prepared 80% ethanol and resuspend the Auto-Mag® D-3 beads by vortex at maximum speed for 20 seconds or pipette mix 20 times.

15. Place the sample tube on the magnet for 2 minutes or until Auto-Mag® D-3 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads

16. Repeat Steps 14-15 for a second 80% ethanol wash.

17. Keep the sample tube on the 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 tube.*

18. Add 30~50 µl Elution Buffer to the sample and resuspend the Auto-Mag® D-3 beads by vortex for 20 seconds or pipette mix 20 times Incubate the sample tube at 55°C for 5 minutes.

19. Place the sample tube back on the magnet for 5 minutes or until the Auto-Mag® D-3 beads are completely cleared from elution buffer.

20. 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 96-Well Format: (96-Well plates with at Least 2 ml Capacity)

1. Prepare the FFPE sample

   1). Prepare the curls from FFPE tissue blocks: Cut sections from FFPE tissue blocks using a microtome. Collect and transfer 1-3 sections into a well of 96-well processing plate with a well capacity of at least 2.0 ml.

   2). Prepare samples from FFPE slides: Scrape the tissue sections in a single direction with a clean razor blade or scalpel, then collect the tissue on the slide into a cohesive mass. Transfer the tissue mass with the scalpel or a pipette tip into a well of 96-well processing plate with a well capacity of at least 2.0 ml.

   Note: *For FFPE slide, 1 cm^2 FFPE sample weight about ~2mg, do not add over 10mg.*

2. Add 500µl FFPE Deparaffinization Reagent (FDR) to each sample. Ensure that the tissues are completely immersed in the FDR.

3. Seal the 96-well plate with sealing film (not provided). Incubate the sample plate at 90°C for 5 minutes to solubilize the paraffin and disperse the tissue. Vortex briefly once during incubation.

4. Remove the plate from the heat source and remove sealing films. Add 200µl FTL Buffer and 20µl Proteinase K to each sample. Seal the plate, gently flip the plate upside down 5 times to disperse the tissue into the FTL buffer.

   Note*: Option: if necessary, Centrifuge plate at 2,000g for 1 minute to create two phases within the solution. When working with multiple samples, FTL buffer, and Proteinase K can be prepared as a mix to save pipetting steps.*

5. Incubate the sample plate at 55°C for 1 Hours. If necessary, extend the incubation time to 4 hours or even overnight to achieve complete lysis, briefly mix by flip plate 2-5 time during incubation.

6. Transfer sample plate to 90°C, incubate for 1 hour for decrosslinking. Then, remove the sample from the heat source and leave at room temperature for 5 minutes,

   Note: Decrosslinking step is strongly recommended for short time lysis (1–4 h) and may be omitted after overnight Lysis at step 5.

7. Option: Remove the sealing film, add 5µl RNase A to each sample. Mix well by repeated pipetting up and down, or pulse vortex or shaking. incubate at room temperature for 5 minutes.

8. Remove the sealing film, add 300µl FDB buffer, 10µl Auto-Mag® D-3, and 300µl 100% isopropanol to each sample, pipette mix 20 times, or seal the 96-well plate and vortex for 20 seconds.

   Note: When working with multiple samples, FDB buffer, and Auto-Mag® D-3, and 100% isopropanol can be prepared as a master mix. Mix well and add 510 for each sample. Prepare only what is needed for each run.

9. Incubate samples at room temperature for 10 minutes,

   Note: There will be two layers, the top layer is FDR with the paraffin, and the bottom layer is an aqueous phase with DNA and magnetic beads. If the paraffin content is too high, there may be some beads that are in the top layer. If this happens, at step 10, keep the sample plate on the magnet, gently shacking the sample plate several times to clear the beads from the top layer.

10. Place the sample plate on a compatible magnetic separation device for 5 minutes or until Auto-Mag® D-3 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads

11. Remove the sample from the magnet. Add 800µl FDW Buffer and resuspend the Auto-Mag® D-3 beads by pipette mix 20 times or seal the 96-well plate and vortex for 20 seconds.

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

12. Place the sample plate on the magnet for 2 minutes or until Auto-Mag® D-3 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads.

13. Repeat Steps 11-12 for a second FDW buffer wash.

14. Remove the sample plate from the magnet. Add 800µl of freshly prepared 80% ethanol and resuspend the Auto-Mag® D-3 beads by pipette mix 20 times or seal the 96-well plate and vortex for 20 seconds.

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15. Place the sample plate on the magnet for 2 minutes or until Auto-Mag® D-3 beads are completely cleared from solution. Remove and discard all the liquid. Do not disturb the attracted beads

16. Repeat Steps 14-15 for a second 80% ethanol wash.

17. Keep the sample plate on the 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 tube.

18. Add 30~50µl Elution Buffer to the sample and resuspend the Auto-Mag® D-3 beads by pipette mix 20 times, or seal the 96-well plate and vortex for 20 seconds. Incubate the sample at 55°C for 5 minutes.

19. Place the sample plate back on the magnet for 5 minutes or until the Auto-Mag® D-3 beads are completely cleared from elution buffer.

20. Transfer the eluate (cleared supernatant) to a new 96-well microplate (not provided). Seal the plate and keep at -20°C for long term storage, or for subsequent applications.

Protocol and Programmed Procedure for Automated Isolation Process

If automating this procedure 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	Tissue was not digested	Ensure that the tissue is fully submerged during the digestion steps.
	Incomplete resuspension of Auto-Mag® D-3.	Resuspend Auto-Mag® D-3 by pipette mixing or vertexing vigorously before use
	Loss of Auto-Mag® D3 during operation.	Avoid disturbing the Auto-Mag® D-3 during aspiration of supernatant.
	FDW Buffer may not be prepared correctly	Prepare buffers accordingly. See "Preparation of Reagents" on Page 3.
	Binding was incomplete	Ensure that the samples are mixed well before collecting the beads. Increase pipette mixing or vortex/shake plate to ensure complete mixing.
Auto-Mag® D-3 do not completely clear from solution	Too short of magnetizing time	Increase collection time on the magnet.
Downstream applications are unsuccessful	DNA is over fixated during tissue formalin fixation	Extend incubation time at 90°C to 90 minutes
Carryover of the magnetic beads in the elution	Bead collection time is too short.	Increase magnetic beads collection time. Residual magnetic particles in eluted DNA will not affect downstream application. Residual magnetic particles in eluted DNA can be magnetized again and the DNA eluate can be transferred to a new storage vessel.

Ordering Information

Product Description	Catalog No.	Size
Auto-Mag® FFPE Tissue DNA Isolation Kit	D008-00 D008-01 D008-02 D008-Bulk	5 Preps. 50 Preps. 200 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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