AMD Biotech Inc.
Advanced Molecular Detection

Product Manual

# Auto-Mag® Soil/Stool DNA Isolation Kit

Version 1.1

Magnetic beads-based kit designed to extract PCR-Ready Genomic DNA from all soil types.

**Catalog Number: D024-00, D024-01, D024-02,**

## 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® Soil/Stool DNA Isolation Kits are designed for the simple and rapid small-scale preparation of highly pure genomic DNA from a wide variety of soil microorganisms, such as bacteria, archaea, fungi, and algae in soil, sludge, and sediment sample. This kit is suitable for samples from forest, bog, farmland, grassland, and stool samples. The kit removes all traces of humic acid and PCR inhibitors using the IHR Reagents (Inhibitor and Humic Acid Removal Reagent). The isolated DNA can be used for most downstream applications such as Southern blot, qPCR, Sanger sequencing and next generation sequencing.

## Features:

- Fast and easy processing using a magnetic bead system.
- Isolate bacterial, fungal, plant and animal genomic DNA from soil and other environmental samples.
- Remove all humic acid and PCR inhibitors from DNA sample.
- high yields of inhibitor-free DNA up to 50 kb and ready for downstream applications.
- Ease of use. Contains glass beads pre-filled in 2 ml disruptor tube.
- Available in a 96-well format that can be integrated with a robotic automation system.

## Kit Contents

| **Product Number** | D024-00 | D024-01 | D024-02 |
|---|---|---|---|
| Preparation | 5 | 50 | 200 |
| Auto-Mag® D-1 | 0.06 ml | 0.6 ml | 2.2 ml |
| SSL Buffer | 4 ml | 30 ml | 120 ml |
| LER Buffer (Lysis Enhancer Reagent) | 0.4 ml | 3 ml | 12 ml |
| IHR Reagent | 0.8 ml | 6 ml | 22 ml |
| IRE Buffer (Inhibitor Remove Enhancer) | 0.8 ml | 6 ml | 22 ml |
| SDB Buffer | 4 ml | 25 ml | 100 ml |
| MDW Buffer * | 3 ml | 30 ml | 120 ml |
| DNA Elution Buffer | 1 ml | 10 ml | 40 ml |
| RNase A | - | 0.3 | 1.1 |
| 2 ml prefilled Disruptor tube ** | 5 | 50 | 200 |
| *** Ethanol must be added prior to use. See Preparation of Reagents**<br>**** Packaged separately** | | | |

## Storage and Stability

Auto-Mag® Soil/Stool DNA Isolation Kit is shipped at room temperature. All components are stable for 12 months after delivery when stored accordingly. Auto-Mag® D-1, IHR Reagent and RNase A should be stored at 2-8°C. SSL Buffer with RNase A mix should be stored at 2-8°C. All other components can be stored at room temperature (15-25°C). Check buffers for precipitates before use. Re-dissolve any precipitates by warming to 37˚C. Do not use after the printed expiration date.

## Preparation of Reagents

1. Add RNase A to the bottle of SLB Buffer before use. Store at 2-8°C

| Reagents | Kit | RNase A to be added |
|---|---|---|
| SSL Buffer | D024-00 | * |
| | D024-01 | 0.3 ml |
| | D024-02 | 1.1 ml |
| * RNase A has already been added to SLB Buffer before shipping.<br>Components are stable for 1 year when stored closed at 2-8°C | | |

2. Dilute MDW Buffer with 100% Ethanol as follows and store at room temperature.

| Reagents | Kit | RNase A to be added |
|---|---|---|
| MDW Buffer | D024-00 | 3 ml |
| | D024-01 | 30 ml |
| | D024-02 | 120 ml |
| Components are stable for 1 year when stored closed at room temperature | | |

3. Prepare 70% Ethanol for DNA Wash, and prepare at least 1 ml for a prep.

## Additional Information

### 1. Specifications

| **Features** | Specification |
|---|---|
| Isolation Technology | Magnetic Beads |
| Sample Sources | Soil, sludge, sediment, and stool |
| Starting Amount | Up to 500 mg |
| Typical Yield | Dependent upon sample, (2-10ug) |
| A260/280 | 1.6-1.9 |
| Elution Volume | 50-100 µl |
| Processing format | Manual |
| Downstream Application | Southern blotting, qPCR, PCR, NGS. |

# Auto-Mag® Soil DNA Isolation Kit protocol

## Materials and Equipment to Be Supplied by User

- Centrifuge
- Magnetic separation device
- Water bath, incubator, or heat block capable of 70°C
- Vortexer
- Ice bucket
- 100% Ethanol
- 70% Ethanol
- Optional: Mixer Mill

## Before Starting

- Please read this booklet in its entirety to become familiar with the procedures.
- Set the incubator to 70°C.
- Prepare an ice bucket and prechill IRE Buffer on ice.
- Prepare SSL buffer, MDW buffer, and 70% Ethanol according to instruction in Preparation Reagents Section.
- Complete resuspension of the Auto-Mag® D-1 by vortex.

## Protocol

1. Add 100-250 mg soil or stool sample to a prefilled 2ml Disruptor tube.

   *Note: The amount of soil sample processed will vary depending on the composition of the sample: process more soil material for wet muddy samples and less for dry sandy samples.*

2. Add 500 µl SSL Buffer to the sample. Vortex sample at maximum speed for 3-5 minutes to lyse and homogenize samples. Alternatively, for best results, use any commercially available bead beater equipment such as Mixer Mill, or Bead blaster to lyse and homogenize samples.

   *Note: RNase A should be added to SSL Buffer before use.*
   *Complete homogenization is critical for best yields.*

3. Add 50µl LER Buffer to the sample. Vortex at maximum speed for 10 seconds. Incubate the sample at 70°C for 10 minutes.

4. Bring sample to room temperature and add 100µl prechilled IHR Reagent and 100µl IRE Buffer. vortex at maximum speed for 20 seconds. Incubate the sample at room temperature for 2 minutes.

   *Note: IHR and IRE Buffer can be prepared as a master mix prior to use. Prepare only what is needed. Completely resuspend IHR Reagent by shaking the bottle before use. If necessary, cut the pipet tip to ease the transfer of IHR Reagent.*

5. Centrifuge sample tube at maximum speeds (≥ 13,000g) at room temperature for 5 minutes and transfer 400 µl supernatant to a new microcentrifuge tube.

   *Note: If supernatant still has a dark color, perform a second IHR Reagent treatment: add additional 100µl prechilled IHR Reagent to the supernatant, vortex to mix thoroughly, centrifuge at maximum speeds for 2 minutes, and transfer 400 µl supernatant to a new microcentrifuge tube. Continue to step 6.*

   *(The additional IHR Reagent (Cat. # AMD-B287) and IRE Buffer (Cat. # AMD-B288) can be purchased separately.)*

6. Add 400µl SDB buffer, 400µl 100% Ethanol, and 10µl Auto-Mag® D-1 to the sample. Vortex at maximum speed for 20 seconds or pipette 20 times. Incubate the sample at room temperature for 5 minutes.

   *Note: Complete resuspension of the Auto-Mag® D-1 before use.*
   *SDB Buffer, Ethanol, and Auto-Mag® D-1 can be prepared as a master mix prior to use. Prepare only when is needed and mix completely.*

7. Place the sample tube on a compatible magnetic separation device 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.

8. Remove the sample tube from the magnet. Add 500µl MDW Buffer and resuspend the Auto-Mag® D-1 beads by vortex at maximum speed for 20 seconds or pipette mix 20 times.

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

9. Optional: Repeat Steps 7-8 for second MDW wash.

10. Remove the sample tube from the magnet. Add 500µl of freshly prepared 70% ethanol and resuspend the Auto-Mag® D-1 beads by vortex at maximum speed for 20 seconds or pipette mix 20 times.

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

12. Repeat Steps 10-11 for second 70% ethanol wash.

13. 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.*

14. Add 50~100µl DNA Elution Buffer to the sample and resuspend the Auto-Mag® D-1 beads by vortex for 20 seconds or pipette mix 20 times. Incubate the sample tube at 55°C for 5 minutes.

15. Place the sample tube back on the 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.

## 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

| Observation | Possible Causes | Comments & Solution |
|---|---|---|
| Wet Soil sample | | Centrifuge sample for 30 seconds at 10,000 x g. Decant most of the liquid, place in the Disruptor Tubes and continue with protocol. |
| Low DNA yield | The sample was not stored properly | Use fresher sample. Ensure that sample are processed immediately after collection or removal from storage. |
| | Poor homogenization of sample | It is important to homogenize the sample thoroughly. Repeat the DNA isolation with a new sample. |
| | Ethanol was not added to the lysate | Ensure ethanol was added to the lysate to bind the DNA to the DNA Binding Beads. |
| | Beads were lost during purification | Avoid disturbing the Auto-Mag® D-1 beads during aspiration of supernatant |
| | DNA washed off | Use 70% ethanol to wash Auto-Mag® D-1. |
| | DNA Not Eluted Efficiently | After resuspending magnetic beads with elution buffer solution, incubate at 55 °C for 5 minutes |
| Problems in downstream applications | Ethanol carry-over | Dry the Auto-Mag® D-1 completely before elution |
| | Non-specific bands in downstream PCR | Further purification of DNA may be necessary, for example, using one of AMD's Auto-Mag® PCR-Pure Kit (Cat # S002) |
| | Excess DNA | Dilute the DNA accordingly |
| Low A260/A230 Ratios for Purified DNA | Ethanol not added to concentrated MDW buffer | Make sure to add 100% Ethanol to concentrated MDW buffer before use. |
| | Proteins not removed efficiently | Repeat with a new sample, be sure to mix the sample with IHR Reagent thoroughly |

## Ordering Information

| Product Description | Catalog No. | Size |
|---|---|---|
| Auto-Mag® Soil/Stool DNA Isolation Kit | D024-00<br>D024-01<br>D024-02<br>D024-Bulk | 5 Preps.<br>50 Preps.<br>200 Prep.<br>Request |

## Related Products and Reagents

| Product Description | Catalog No. | Size |
|---|---|---|
| IHR Reagent | B287-00<br>B287-01<br>B287-02<br>B287-Bulk | 1 ml<br>6 ml<br>22 ml<br>Request |
| IRE Buffer | B288-00<br>B288-01<br>B287-02<br>B288-Bulk | 1 ml<br>6 ml<br>22 ml<br>Request |
| 2 ml Prefilled Tissue Disruptor Tube | B012-00<br>B012-01<br>B012-02<br>B012-Bulk | 5<br>50<br>200<br>Request |

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