AMD Biotech Inc.
Advanced Molecular Detection

Product Manual

Auto-Mag® Agarose Gel DNA Extraction Kit

Version 1.1

Magnetic bead-based chemical for Agarose gel DNA extraction

Catalog Number: S010-00, S010-01, S010-02,

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® Agarose Gel DNA Extraction Kit uses specially coated magnetic beads and the corresponding buffer system that provides rapid purification of high-quality DNA from TAE/TBE-buffered agarose gels. The process does not involve toxic reagents and is able to recover DNA fragments of 100 bp to 20 kb in size with yields exceeding 80%. Eluted DNA is suitable for ligations, PCR, sequencing, restriction digestion, or various labeling reactions.

The kit also provides the procedure for DNA Extraction by using 1.5 ml tubes and alternative procedure for DNA Extraction without using magnetic separation devices.

Features:

- Quick recovery of ultra-pure DNA fragments from agarose gels
- Highly specific binding of DNA allows easy removal of impurities and inhibitors
- No need for centrifugation or filtration in standard extraction procedure.
- Eluted DNA is well suitable for use in DNA ligation, sequencing, labeling, PCR, etc.

Kit Contents

Product Number	S010-00	S010-01	S010-02
Preparation	5	50	200
Auto-Mag® D-1	0.06 ml	0.6 ml	2.2 ml
GLB Buffer	4 ml	40 ml	160 ml
GDW Buffer *	3 ml	30 ml	120 ml
Elution Buffer	1 ml	10 ml	40 ml
* Ethanol must be added prior to use. See Preparation of Reagents			

Storage and Stability

Auto-Mag® Agarose Gel DNA Extraction Kit is shipped at room temperature. All components are stable for 12 months after delivery when stored accordingly. Auto-Mag® D-1 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). 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. Dilute GDW Buffer with 100% Ethanol as follows and store at room temperature.

Reagents	Kit	100% Ethanol to be Added
GDW Buffer	S010-00	3 ml
	S010-01	30 ml
	S010-02	120 ml
Components are stable for 1 year when stored closed at room temperature		

2. Prepare 70% ethanol for DNA wash steps and prepare at least 1.0 ml per test.

Additional Information

1. Specification

Features	Specification
Isolation Technology	Magnetic Beads
Sample Sources	Agarose gel
Starting Amount	Up to 500 mg
Typical Yield	Dependent upon sample
A260/280	1.6-1.9
Elution Volume	30-50µl
Processing format	Manual
Downstream Application	ligations, PCR, sequencing, restriction digestion, qPCR, PCR, NGS

Auto-Mag® Agarose Gel DNA Extraction Protocol

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.
- Vortexer
- Incubator capable of 60°C
- 100% Ethanol
- 70% Ethanol

Before Starting

- Ensure GDW Buffer is prepared according to the instructions of Preparation Reagents on page 3.
- Prepare 70% ethanol for final DNA wash steps.
- Preset water bath, incubator, or heating blocks to 60°C.
- Complete resuspension of the Auto-Mag® D-1 beads by vortex

Protocol for Agarose Gel DNA Extraction

1. Follow the Agarose Gel Electrophoresis Protocol to run gel electrophoresis for the DNA samples.

   Note: Gel purification is most efficient with lower % agarose gels. To stay in the 0.7-0.8% range if possible. It is strongly recommended to use fresh TAE buffer or TBE buffer as running buffer. Do not reuse running buffer as its pH will increase and reduce yields.

2. After gel electrophoresis, move the gel from gel tray to an open UV box that is lined with a plastic film, as it will block some of the UV, and with a clean, sterile razor blade, slice the desired DNA fragment from the gel.

   Note: Be sure to wear proper UV protection - especially for your eyes! Try to get as little excess gel around the DNA band as possible.

3. Place the gel in a labeled microfuge tube. Using a scale, weigh the tube with the gel fragment after zeroing the scale with an empty tube.

   Note: The weight of the gel is directly proportional to its liquid volume, and a gel slice of mass 0.1 g will have a volume of 0.1 ml.

4. Add 2x gel volume of GLB Buffer to the sample. Incubate sample tube at 60°C for 10 minutes or until the gel has completely melted. Vortex or shake the sample tube every 2-3 minutes.

   Note: For example: A gel slice of mass is 0.2 g, add 0.4ml GLB Buffer.

5. Cool sample at room temperature, add 10µl Auto-Mag® D-1 and 1x gel volume of 100% Ethanol to each sample and pipette mix 20 times or vortex for 20 seconds. Incubate the sample at room temperature for 10 minutes.

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

6. Place the samples tube on a compatible magnetic separation device 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.

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

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

8. Place the sample tube on the magnet for 2 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.

9. Repeat Steps 7-8 for a second GDW buffer wash.

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

11. Place the sample tube on the magnet for 2 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 or optional third 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 30-50µl DNA Elution Buffer to the sample and resuspend the Auto-Mag® D-1 by pipette mix 20 times or vortex for 20 seconds. Incubate the sample tube at 60°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.

Alternative Protocol for Using Centrifuge Method

1. Follow the Agarose Gel Electrophoresis Protocol to run gel electrophoresis for the DNA samples.

   Note: Gel purification is most efficient with lower % agarose gels. To stay in the 0.7-0.8% range if possible. It is strongly recommended to use fresh TAE buffer or TBE buffer as running buffer. Do not reuse running buffer as its pH will increase and reduce yields.

2. After gel electrophoresis, move the gel from gel tray to an open UV box that is lined with a plastic film, as it will block some of the UV, and with a clean, sterile razor blade, slice the desired DNA fragment from the gel.

   Note: Be sure to wear proper UV protection - especially for your eyes! Try to get as little excess gel around the DNA band as possible.

3. Place the gel in a labeled microfuge tube. Using a scale, weigh the tube with the gel fragment after zeroing the scale with an empty tube.

   Note: The weight of the gel is directly proportional to its liquid volume, and a gel slice of mass 0.1 g will have a volume of 0.1 ml.

4. Add 2x gel volume of GLB Buffer to the sample. Incubate sample tube at 60°C for 10 minutes or until the gel has completely melted. Vortex or shake the sample tube every 2-3 minutes.

   Note: For example: A gel slice of mass is 0.2 g, add 0.4ml GLB Buffer.

5. Cool sample at room temperature, add 10µl Auto-Mag® D-1 and 1x gel volume of 100% Ethanol to each sample and pipette mix 20 times or vortex for 20 seconds. Incubate the sample at room temperature for 10 minutes.

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

6. Centrifuge the sample tube at 5,000rpm (or 2,300 x g) for 1 minute to pellet the magnetic bead and discard the supernatant by pipetting. Do not disturb the beads.

   Note: Ensure that the speed of the centrifuge does not exceed 5,000rpm (or 2,300 x g).

7. Add 500µl GDW Buffer and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

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

8. Centrifuge the sample tube at 5,000rpm (or 2,300 x g) for 1 minute to pellet the magnetic bead and discard the supernatant by pipetting. Do not disturb the beads.

9. Repeat Steps 7-8 for a second GDW buffer wash.

10. Add 500µl 70% ethanol and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

11. Centrifuge the sample tube at 5,000rpm (or 2,300 x g) for 1 minute to pellet the magnetic bead and discard the supernatant by pipetting. Do not disturb the beads.

12. Repeat Steps 9-10 for second 70% ethanol wash or optional third 70% ethanol wash.

13. Remove any residual liquid with a pipette and air dry the magnetic beads at room temperature for 5 minutes.

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

14. Add 30-50µl DNA Elution Buffer to the sample and resuspend the Auto-Mag® D-1 by pipette mix 20 times or vortex for 20 seconds. Incubate the sample tube at 60°C for 5 minutes.

15. Centrifuge the sample tube at 12,000 x g for 2 minutes to pellet the magnetic beads tightly

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

Symptoms	Possible Causes	Comments
Low DNA Yields	Incomplete solubilization of the gel slice	Verify that a 1:2 volume of GLB Buffer is added to a precisely weighted gel slice (for every 100 mg of agarose gel, add 100µl of GLB Buffer).
	Inefficient DNA binding	Check the color of the solution after the gel slice is completely dissolved. A yellow color indicates an optimal pH for DNA binding. If the solution color is orange or violet, add 10µl of 3 M sodium acetate, pH 5.2 solution and mix. The color of the mix will become yellow.
	Inefficient DNA elution	DNA elution is dependent on pH, temperature, and time. For large genomic DNA (≥50 kb), apply heated elution buffer to 60°C or Incubate at 60°C to increase yield.
	Magnetic Beads	Make sure to re-suspend Auto-Mag® D-1 completely before use. Beads can settle out quickly in solution.
	Ethanol is not added into GDW Buffer	Add absolute 100% Ethanol to GDW (see Page 3 for instructions)
Low quality results	Contamination from reused electrophoresis buffer	If extracted DNA is used directly for sequencing, freshly prepared electrophoresis buffers should be used both for gel preparation and for gel running.
Downstream applications are unsuccessful	Presence of residual ethanol	Ensure to air dry the Auto-Mag® C-5. Remove any residual liquid with a pipette.
	Eluate contaminated with agarose	Ensure the gel slice is properly solubilized during steps 1-2. Verify that a 1:2 volume of GLB Buffer was added to a precisely weighted gel slice. Large amounts of agarose or agarose gel percentages greater than 2% may take more time to dissolve. In some cases, adding a larger volume of GLB Buffer and vortexing the gel solution more frequently can facilitate solubilization

	Eluate contaminated with excess salt.	Ensure that the wash is effective. Incubate Auto-Mag® D-1 with the Wash Buffer for several minutes before proceeding to magnetic separation.

Ordering Information

Product Description	Catalog No.	Size
Auto-Mag® Gel DNA Extraction Kit	S010-00 S010-01 S010-02 S010-Bulk	5 Preps. 50 Preps. 200 Preps. Request

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