AMD Biotech Inc.
Advanced Molecular Detection

Product Manual

Auto-Mag® Plasmid DNA Miniprep Kit

Version 2.1

Magnetic beads-based kit designed to extract high-quality plasmid DNA from bacterial cultures.

Catalog Number: D014-00, D014-01, D014-02,

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.

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Product Introduction

Auto-Mag® Plasmid DNA Miniprep Kit utilized magnetic beads with alkaline-SDS lysis of bacterial cells to isolate high-quality plasmid DNA in a high-throughput format. By using a 96-well format, up to 96 samples can be simultaneously processed in less than 60 minutes. By utilizing magnetic beads chemistry, the system is geared for automation as the protocol requires no vacuum filtration. When used with high copy plasmids, Auto-Mag® Plasmid DNA Miniprep Kit yields ~5-15µg plasmid. Yields vary slightly depending on the cell line, vector type, and size of the construct. Purified plasmid DNA can be used in downstream applications directly.

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

Features:

- Fast, reproducible, and easy processing using a magnetic bead system
- High quality plasmid DNA purification
- Recovered plasmid DNA is compatible with various downstream applications
- Adaptable to various automated liquid handling workstations
- No toxic organic solvents

Kit Contents

Product Number	**D014-00**	**D014-01**	**D014-02**	**D014-03**
Preparation	5	96	384	1920
Auto-Mag® D-1	0.06 ml	1.1 ml	4.2 ml	21 ml
Solution 1*	1 ml	10 ml	40 ml	200 ml
Solution 2	1 ml	10 ml	40 ml	200 ml
Neutralization Buffer	1 ml	10 ml	40 ml	200 ml
Elution Buffer	1 ml	10 ml	40 ml	200 ml
RNase A	*	0.1 ml	0.4 ml	2.0 ml
* RNase A must be added prior to use. See Preparation of Reagents				

Storage and Stability

Auto-Mag® Plasmid DNA Miniprep Kit is shipped at ambient temperature. All components are stable for 12 months when stored accordingly. Auto-Mag® D-1 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. Solution I/RNase A mixture should be stored at 2-8°C. All remaining 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. Add RNase A to the bottle of Solution I before use. Store at 2-8°C

Reagents	Kit	RNase A to be added
Solution 1	D014-00	*
	D014-01	0.1 ml
	D014-02	0.4 ml
	D014-03	2.0 ml
* RNase A has already been added to Solution 1 before shipping. Components are stable for 1 year when stored closed at 2-8°C.		

2. Prepare 70% Ethanol for plasmid DNA Wash, and prepare at least 1.2 ml for a prep.

Additional Information

1. Bacterial Strain Selection

- It is strongly recommended that an end A negative strain of E. coli be used for routine plasmid isolation. Examples of such strains include DH5α, DH1, and C600. These host strains yield high-quality Plasmid DNA with Auto-Mag® Plasmid DNA Kits' Protocols.

- Host strains derivatives from HB101 such as TG1 and the JM100 series release large amounts of carbohydrates during lysis, which may inhibit enzyme activities when not completely removed. Some strains may also lower DNA quality due to having high levels of endonuclease activity, and therefore are not recommended (i.e. JM101, JM110, HB101).

2. Bacterial Culture Growth

- Bacterial cultures for plasmid preparations should always be grown from a single colony picked from a freshly streaked plate. Subculturing directly from glycerol stock or liquid cultures may lead to uneven yields or plasmid loss. Optimal results are obtained by using one single isolated colony from a freshly transformed or freshly streaked plate to inoculate an appropriate volume of starter culture containing the appropriate antibiotic, and then incubated for 12-16 at 37°C with vigorous shaking (~250 rpm, shaking incubator). Aeration is very important. The culture volume should not exceed 1/4 the volume of the container.

- Auto-Mag® Plasmid DNA Prep Kits are specially designed for use with cultures grown in Luria Bertani (LB) medium. Richer broths such as TB (Terrific Broth) or 2 x YT led to high cell densities that can overload the purification system, and therefore are not recommended. If rich media must be used, growth times should be optimized, and the recommended culture volumes may be reduced to match the capacity of the Auto-Mag® D-1 beads or use more beads.

- For optimal plasmid yields, the starting culture volume should be based on culture cell density. A bacterial density between 2.0 and 3.0 at OD600 is recommended. When using nutrient-rich media, care should be taken to ensure that the cell density does not exceed an OD600 of 3.0. Using a high-density culture outside of the recommended OD range may overload the purification system.

3. Harvest the Bacteria

- Harvest cells by centrifugation at 10,000 × g for 10 min. Discard the supernatant. The bacterial pellet can be used immediately or stored at -20°C.

4. Specifications

Features	Specification
Isolation Technology	Magnetic Beads
Sample Sources	E. coli LB cultures
Starting Amount	Up to 1.5 ml
Typical Yield	Dependent upon sample, (2-15 µg)
A260/280	1.6-1.9
Elution Volume	50-100 µl
Processing format	Automated; Manual
Downstream Application	Cloning, qPCR, Real-Time PCR, NGS. etc.

Auto-Mag® Plasmid DNA Miniprep 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.
- 96-well format: 500µl round bottom microtiter plate and compatible magnetic separation device for 96-well plate
- Centrifuge with swing-bucket rotor capable of 5,000 x g for 96-well well plates
- Rotor for 96-well deep-well plates
- 2.0 ml 96-deepwell Plate for bacterial growth
- Destination Plate: >500 round bottom microtiter plate
- Sealing film for 96-well plant processing
- Incubator/water bath capable of 55°C
- 100% Ethanol
- 70% Ethanol

Before Starting

- Ensure Solution 1 is prepared according to the instructions of Preparation Reagents on page 3.
- Prepare 70% ethanol for Plasmid DNA wash steps.
- Preset water bath, incubator, or heating blocks to 55°C.
- Pre-chill Neutralization Buffer to 4°C.
- Complete resuspension of the Auto-Mag® D-1 by vortex.

Protocol for 96-Well Format

1. Inoculate 1-1.5 mL LB/antibiotic(s) medium with E. coli in a 96-well deep-well plate and incubate at 37°C with agitation for 12-16 hours.
2. Pellet bacteria by centrifuging the culture plate at 3,000 x g for 10 minutes.
3. Discard supernatant. Dry the plate by inverting the plate on an absorbent paper towel to remove excess media.
4. Add 100µl of Solution 1/RNase A. and thoroughly resuspend the cell pellets by pipetting up and down 20 times or

pulse vortexing. Incubation at room temperature for 5 minutes. The mixture should appear homogenous and should not have any cell clumps.

Note: RNase A must be added to Solution I before use.

5. Add 100µl of Solution 2 to the cell suspension, gently mix by shaking and rotating the plate for 1 minute to obtain a clear lysate, Incubation at room temperature for 5 minutes with occasional mixing.

   Note： Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution 2 tightly capped when not in use to avoid acidification from CO2 in the air.

6. Add 100µl of chilled (4°C) Neutralization Buffer, and gently mix by shaking until a flocculent white precipitate forms. Incubate at room temperature for 10 minutes to complete the neutralization.

7. Centrifuge the samples plate at 5,000 x g for 10 minutes to pellet the flocculent material. Transfer ~200µl of the clear lysate to a 500µl round bottom microtiter plate.

   Note: The transfer of the clear lysate is a critical step of the process. The supernatant should be free of flocculent material for optimal results.

8. Add 10µl of Auto-Mag® D-1 and 200µl of 100% Ethanol to each well. Mix immediately by pipette mix 20 times to completely resuspend samples.

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

9. Incubate the plate at room temperature for 5 minutes with occasional mixing. Or shake the sample on an orbital shaker at 200-600 RPM for the entire duration of incubation.

   Note: For low copy number plasmid, longer incubation may increase yield.

10. Place the sample plate 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.

    Note: The supernatant may have a slight yellow-brown tint but should not be cloudy.

11. Remove the sample plate from the magnet. Add 400µl 70% ethanol and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

12. Place the sample plate 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.

13. Repeat Steps 11-12 for a second and third wash.

14. 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 well.

15. 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.

16. Incubate the sample plate at 55°C for 5 minutes.

17. Place the sample plate back on the magnet for 5 minutes or until the Auto-Mag® D-1 beads are completely cleared from elution buffer.

18. 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 Using Single 1.5-2.0 ml Tube

1. Inoculate 1-1.5 mL LB/antibiotic(s) medium with E. coli at 37°C with agitation for 12-16 hours.

2. Pellet bacteria by centrifuging at 10,000 x g for 2 minutes.

3. After centrifugation, carefully remove and discard the supernatant. Do not disturb or dislodge the cell pellet.

4. Add 100µl of Solution 1/RNase A. and thoroughly resuspend the cell pellets by pipetting up and down 20 times or pulse vortexing. Incubation at room temperature for 5 minutes. The mixture should appear homogenous and should not have any cell clumps.

   Note: RNase A must be added to Solution I before use.

5. Add 100µl of Solution 2 to the cell suspension, gently mix by inverting the tube for 1 minute to obtain a clear lysate, Incubation at room temperature for 5 minutes with occasional mixing.

   Note： Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution 2 tightly capped when not in use to avoid acidification from CO2 in the air.

6. Add 100µl of chilled (4°C) Neutralization Buffer, and gently mix by shaking until a flocculent white precipitate forms. Incubate at room temperature for 10 minutes to complete the neutralization.

7. Centrifuge the samples plate at 5,000 x g for 10 minutes to pellet the flocculent material. Transfer ~200µl of the clear lysate to a 500µl round bottom microtiter plate.

   Note: The transfer of the clear lysate is a critical step of the process. The supernatant should be free of flocculent material for optimal results.

8. Add 10µl of Auto-Mag® D-1 and 200µl of 100% Ethanol to each well. Mix immediately by pipette mix 20 times to completely resuspend samples.

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

9. Incubate the sample at room temperature for 5 minutes with occasional mixing. Or shake the sample on an orbital shaker at 200-600 RPM for the entire duration of incubation.

   Note: For low copy number plasmid, longer incubation may increase yield.

10. 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.

    Note: The supernatant may have a slight yellow-brown tint but should not be cloudy.

11. Remove the sample tube from the magnet. Add 400µl 70% ethanol and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

12. 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.

13. Repeat Steps 11-12 for a second and third wash.

14. 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 well.

15. 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.

16. Incubate the sample tube at 55°C for 5 minutes.

17. 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.

18. 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 1.5 ml Tube and Centrifuge

1. Inoculate 1-1.5 mL LB/antibiotic(s) medium with E. coli at 37°C with agitation for 12-16 hours.

2. Pellet bacteria by centrifuging at 10,000 x g for 2 minutes.

3. After centrifugation, carefully remove and discard the supernatant. Do not disturb or dislodge the cell pellet.

4. Add 100µl of Solution 1/RNase A. and thoroughly resuspend the cell pellets by pipetting up and down 20 times or pulse vortexing. Incubation at room temperature for 5 minutes. The mixture should appear homogenous and should not have any cell clumps.

   Note: RNase A must be added to Solution I before use.

5. Add 100µl of Solution 2 to the cell suspension, gently mix by inverting the tube for 1 minute to obtain a clear lysate, Incubation at room temperature for 5 minutes with occasional mixing.

Note：Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution 2 tightly capped when not in use to avoid acidification from CO2 in the air.

6. Add 100µl of chilled (4°C) Neutralization Buffer, and gently mix by shaking until a flocculent white precipitate forms. Incubate at room temperature for 10 minutes to complete the neutralization.

7. Centrifuge the samples plate at 5,000 x g for 10 minutes to pellet the flocculent material. Transfer ~200µl of the clear lysate to a 500µl round bottom microtiter plate.

   Note: The transfer of the clear lysate is a critical step of the process. The supernatant should be free of flocculent material for optimal results.

8. Add 10µl of Auto-Mag® D-1 and 200µl of 100% Ethanol to each well. Mix immediately by pipette mix 20 times to completely resuspend samples.

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

9. Incubate the sample at room temperature for 5 minutes with occasional mixing. Or shake the sample on an orbital shaker at 200-600 RPM for the entire duration of incubation.

   Note: For low copy number plasmid, longer incubation may increase yield.

10. 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).

11. Add 400µl 70% ethanol and resuspend the Auto-Mag® D-1 beads by pipette mix 20 times or vortex for 20 seconds.

12. Centrifuge the sample tube at 5,000rpm (or 2,300 x g) for 1 minute to pellet the magnetic beads 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).

13. Repeat Steps 11-12 for a second and third wash.

14. 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 well.

15. 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.

16. Incubate the sample tube at 55°C for 5 minutes.

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

18. 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 yield	Plasmid did not propagate	Ensure that the appropriate growth medium, supplements, and antibiotics were used for the host cell and plasmid of interest.
	Poor cell lysis	Do not use more than 1ml with high copy plasmids.
		Cells may not be dispersed adequately prior to addition of Solution 1. Vortex cell suspension to completely disperse.
		Increase incubation time with Solution 2 to obtain a clear lysate.
		Solution 2 needs to store tightly. If not, it may need to be replaced.
	Bacterial culture overgrown or not fresh	Do not incubate cultures for more than 16 hours at 37°C
	Low copy-number plasmid used	Such plasmids may yield as little as 0.1µg DNA from a 1 ml overnight culture.
High-molecular weight DNA contamination	Over mixing of cell lysate upon addition of Solution 2	Do not vortex or aggressively mix after adding Solution 2
Optical densities do not agree with DNA yield on agarose gel	Trace contaminants eluted from beads increase A260	Make sure to wash the Auto-Mag® D-1 as instructed. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantification
RNA visible on agarose gel	RNase A not added to Solution 1	Prepare Solution 1 as instructed on Preparation of Reagents section.
DNA does not perform well in Downstream application	DNA was not washed with 70% Ethanol (freshly prepared)	Traces of salt from the binding step may remain in the sample if the magnetic beads are not washed with 70% Ethanol (freshly prepared). Salt may interfere with downstream applications, and thus must be washed from the magnetic beads
	A different elution buffer was used	If a different elution buffer other than the one provided in the kit was used, the buffer should be checked for any components that may interfere with the application.

Ordering Information

Product Description	Catalog No.	Size
Auto-Mag® Plasmid DNA Miniprep Kit	D014-00 D014-01 D014-02 D014-03 D014-Bulk	5 Preps. 50 Preps. 200 Prep. 2,000 Preps Request

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