Auto-Mag® Plasmid DNA Midiprep Kit

Version 2.1

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

Catalog Number: D016-00, D016-01, D016-02,

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 Midi Prep Kit is magnetic beads-based plasmid purification system and designed for large scale isolation of high-quality plasmid DNA from recombinant E. coli cultures. Up to 80-250µg high copy number plasmid DNA or 10-50µg low copy number plasmid DNA can be purified from 10-50 ml overnight culture using the Auto-Mag® Plasmid DNA Midiprep Kit. Purified plasmid DNA can be used in a wide variety of downstream applications directly, such as fluorescent DNA sequencing, restriction endonuclease digestion, PCR, cloning, transformation, transfection of mammalian cells, and other manipulations.

The kit also provides the 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	**D016-00**	**D016-01**	**D016-02**
Preparation	2	25	100
Auto-Mag® D-1	0.4 ml	6 ml	24 ml
Solution 1*	10 ml	128 ml	510 ml
Solution 2	10 ml	128 ml	510 ml
Neutralization Buffer	10 ml	128 ml	510 ml
Elution Buffer	10 ml	128 ml	510 ml
RNase A	0.1 ml	1.28 ml	5.1 ml
* RNase A must be added prior to use. See Preparation of Reagents			

Storage and Stability

Auto-Mag® Plasmid DNA Midi Prep 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	D016-00	0.1 ml
	D016-01	1.28 ml
	D016-02	5.1 ml
* Components are stable for 1 year when stored closed at 2-8°C.		

2. Prepare 70% Ethanol for plasmid DNA Wash.

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 50 ml
Typical Yield	Dependent upon sample
A260/280	1.6-1.9
Elution Volume	Dependent upon sample
Processing format	Manual
Downstream Application	Cloning, qPCR, Real-Time PCR, NGS. etc.

Auto-Mag® Plasmid DNA Midi Prep Protocol

Materials and Equipment to Be Supplied by User

- Nuclease-free 15 ml and 50 ml centrifuge tubes
- Centrifuge with capable of 12,000 x g
- 30 ml or 50 ml centrifuge tubes capable of withstanding 15,000g
- vortex
- 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 Midi Prep Protocol (10-50ml sample volume)

This protocol as written is for processing a different volume of cultures, please check Table 1 to determine the reagents volume used for plasmid isolation. All centrifugation steps should be carried out at room temperature unless otherwise.

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

2. Transfer 10-50ml overnight culture to an appropriate volume centrifuge tube, centrifuge at 10,000 x at room temperature for 10 minutes to collect bacteria.

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

Note: To ensure that all traces of the medium are removed, use a clean paper towel to blot excess liquid from the wall of the tube.

4. Refer to Table 1, based on the sample volume, add the correct volume of Solution 1/RNase A to sample and vortex

or pipet up and down to completely resuspend the cells. The mixture should appear homogenous and should not have any cell clumps.

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

Table 1: List of volumes of reagents to be added for Lysis step, Binding step, and Wash /Elution step based on the sample volumes.

Original Sample Vol. (ml)	Lysis step			Binding step				Wash and Elution steps	
	Solution 1 (ml)	Solution 2 (ml)	Neutralization Buffer (ml)	Supernatant (ml)	Auto-Mag® D-1 (µl)	Add 100% Ethanol (ml)	Binding (min)	70% Ethanol (ml)	Elution (ml)
10~15	1.5	1.5	1.5	4.5	75	4.5	15	10x3	0.75-1.5
16~30	3	3	3	9	150	9	20	20x3	1.5-3.0
31~50	5	5	5	15	200	15	25	25x3	2.5-5.0

5. Refer to Table 1, based on the sample volume, add the correct volume of Solution 2 to the cell suspension, Invert and rotate the tube gently 8-10 times to obtain cleared 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. Refer to Table 1, based on the sample volume, add the correct volume of chilled (4°C) Neutralization Buffer, and mix thoroughly and immediately by invert and rotate the tube gently until flocculent white precipitates form. Incubate at room temperature for 10 minutes to complete the neutralization.

7. Centrifuge at 10,000g for 10 minutes at room temperature (preferably at 4°C). A compact white pellet will form. Transfer all the clear lysate to a new tube. Be careful not to disturb the pellet and that no cellular debris is transferred.

   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. Refer to Table 1, based on the sample volume, add the correct volume of 100% Ethanol and Auto-Mag® D-1 to The clear lysate. Mix immediately by vortex or pipet up and down to completely resuspend samples.

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

9. Refer to Table 1, based on the sample volume, Incubate the sample at room temperature for 15-25 minutes. During incubation, vortex the sample for 10 seconds every 5 minutes. 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 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.

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

11. Remove the sample tube from the magnet. Refer to Table 1, based on the sample volume, add correct volume 70% ethanol and resuspend the beads by vortex or pipet up and down to completely resuspend beads.

12. Place the sample tube 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.

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

14. Keep the sample tube on the magnet, and air-dry Auto-Mag® D-1 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 sample tube.

15. Refer to Table 1, based on the sample volume, add an appropriate volume of Elution Buffer to the sample and completely resuspend Auto-Mag® D-1 beads by vortex for 30 seconds.

Note: For ease of elution and incubation steps, it is recommended to transfer beads/elution buffer mixture to a smaller tube for heating incubation.

16. Incubate the sample 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 without using magnetic separation devices.

All centrifugation steps should be performed with a swing bucket rotor for maximum plasmid DNA yields. All centrifugation steps should be carried out at room temperature unless otherwise noted.

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

2. Transfer 10-50ml overnight culture to an appropriate volume centrifuge tube, centrifuge at 10,000 x at room temperature for 10 minutes to collect bacteria.

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

Note: To ensure that all traces of the medium are removed, use a clean paper towel to blot excess liquid from the wall of the tube.

4. Refer to Table 1, based on the sample volume, add the correct volume of Solution 1/RNase A to sample and vortex or pipet up and down to completely resuspend the cells. The mixture should appear homogenous and should not have any cell clumps.

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

Table 1: List of volumes of reagents to be added for Lysis step, Binding step, and Wash /Elution step based on the sample volumes.

Original Sample Vol. (ml)	Lysis step			Binding step				Wash and Elution steps	
	Solution 1 (ml)	Solution 2 (ml)	Neutralization Buffer (ml)	Supernatant (ml)	Auto-Mag® D-1 (µl)	Add 100% Ethanol (ml)	Binding (min)	70% Ethanol (ml)	Elution (ml)
10~15	1.5	1.5	1.5	4.5	75	4.5	15	10x3	0.75-1.5
16~30	3	3	3	9	150	9	20	20x3	1.5-3.0
31~50	5	5	5	15	200	15	25	25x3	2.5-5.0

5. Refer to Table 1, based on the sample volume, add the correct volume of Solution 2 to the cell suspension, Invert and rotate the tube gently 8-10 times to obtain cleared 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. Refer to Table 1, based on the sample volume, add the correct volume of chilled (4°C) Neutralization Buffer, and mix thoroughly and immediately by invert and rotate the tube gently until flocculent white precipitates form. Incubation at room temperature for 10 minutes to complete the neutralization.

7. Centrifuge at 10,000g for 10 minutes at room temperature (preferably at 4°C). A compact white pellet will form. Transfer all the clear lysate to a new tube. Be careful not to disturb the pellet and that no cellular debris is transferred.

   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. Refer to Table 1, based on the sample volume, add the correct volume of 100% Ethanol and Auto-Mag® D-1 to The clear lysate. Mix immediately by vortex or pipet up and down to completely resuspend samples.

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

9. Refer to Table 1, based on the sample volume, Incubate the sample at room temperature for 15-25 minutes. During incubation, vortex the sample for 10 seconds every 5 minutes. 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 5 minute to pellet the magnetic bead and discard the all the liquid. Do not disturb the beads.

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

11. Remove the sample tube from the magnet. Refer to Table 1, based on the sample volume, add correct volume 70% ethanol and resuspend the beads by vortex or pipet up and down to completely resuspend beads.

12. Centrifuge the sample tube at 5,000rpm (or 2,300 x g) for 5 minute to pellet the magnetic bead and discard the all the liquid. Do not disturb the beads.

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 sample tube.

15. Refer to Table 1, based on the sample volume, add an appropriate volume of Elution Buffer to the sample and completely resuspend Auto-Mag® D-1 beads by vortex for 30 seconds.

    Note: For ease of elution and incubation steps, it is recommended to transfer beads/elution buffer mixture to a smaller tube for heating incubation.

16. Incubate the sample 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	Reduce the initial volume of culture or increase the lysis time while monitoring the lysis visually.
		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	Double culture volume
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 Midi Prep Kit	D016-00 D016-01 D016-02 D016-Bulk	5 Preps. 25 Preps. 100 Prep. Request

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