Application Note

Optimizing Plasmid Yield with the AmMag™ Quatro 1400 with Non-separation Elution Protocol

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Introduction

The AmMag™ Quatro 1400 is a fully automated, advanced magnetic bead–based system designed for midi to maxi-scale purification of high-quality, transfection-grade plasmid DNA from *Escherichia coli* cultures. It consistently delivers high yields of plasmid DNA with purity suitable for sensitive downstream applications, including in vitro transcription (IVT), transfection, cloning, and sequencing. Built to meet the growing demands of high-throughput molecular biology workflows, the Quatro 1400 ensures reliable scalability, minimal hands-on time, and consistent performance, making it an ideal solution for both research and production environments.

The Quatro 1400 system typically uses a separation elution method (Figure 1A), where magnetic beads are held in the reaction tube by a magnet, and only the bead-free supernatant is transferred to the final collection tube. While effective, this method can lead to up to 40% volume loss, as residual liquid remains bound to the beads—resulting in a similar reduction in plasmid DNA yield.

To address this limitation, we implemented a non-separation elution method (Figure 1B). In this approach, the entire slurry, including the magnetic beads, is transferred to the collection tube. The beads are subsequently separated from the plasmid solution via centrifugation through an integrated 0.22 µm filter.

This method significantly improves volume recovery, as nearly the entire elution buffer volume is collected—unlike in separation elution, where up to 40% of the buffer can remain trapped with the beads, reducing the final volume and yield. As a result, the total plasmid yield increases with non-separation elution.

Importantly, the plasmid concentration remains comparable between the two methods, since increased yield is primarily due to greater recovery of eluted volume, not dilution or changes in elution efficiency. This makes the non-separation method a practical way to boost DNA recovery without compromising concentration or purity.

In this application note, we evaluate the non-separation elution strategy using the pcDNA3.1(+) plasmid, focusing on both plasmid yield and quality across varying binding bead and elution volumes.

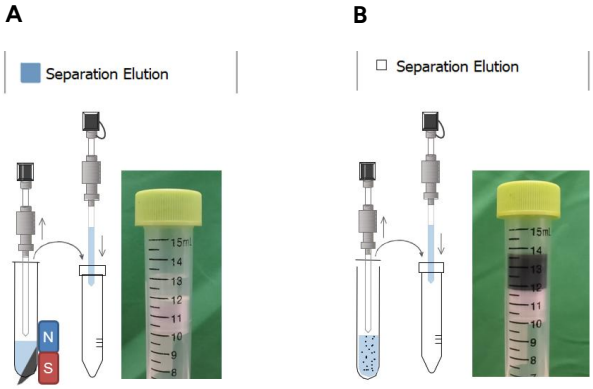

Figure 1. Comparison of separation and non-separation elution methods. (A) Selected option on the Quatro controller and schematic representation of the separation elution method and a sample collection tube showing the sample after the run using the separation protocol. (B) Selected option on the Quatro controller and schematic representation of the non-separation elution method and a sample collection tube showing the sample after the run using the non-separation protocol.

Materials and Methods

Transformation

The pcDNA3.1(+) plasmid (GenScript) containing an ampicillin resistance gene was transformed into DH5α competent *E. coli* cells according to the manufacturer's protocol. The transformed cells, were plated on an LB agar plate with 100 µg/mL ampicillin and incubated at 37°C for 16 h.

Bacterial Culture

A single colony was picked from the plate and inoculated into 5mL started culture containing LB medium supplemented with 100 µg/mL ampicillin. The culture was grown at 37°C with shaking at 220 rpm for 8 hours in a 25 mm orbital shaker (Eppendorf Innova® 40).

Next, 500 µL of the starter culture was inoculated into 500 mL of Terrific Broth (TB) medium, containing 100 µg/mL ampicillin, in a 2 L culture flask. The flasks were incubated at 37°C with shaking at 220 rpm for 16 hours in a 25 mm orbital shaker (Eppendorf Innova® 40).

Pellet preparation

To harvest the cells, the resulting culture was combined and distributed into Quatro sample collection tubes, each containing approximately 40 mL. The tubes were centrifuged at 4,000 × g for 15 minutes at 4°C. After centrifugation, the cell pellets were weighed and adjusted to 1–1.5 g for "High" pellets by centrifuging additional culture volume into the same tubes, before being frozen and stored at –20°C for further use.

DNA Purification

Purification of the pcDNA3.1(+) plasmid was carried out using the AmMag™ Quatro 1400 with the standard GenScript kit (L00882-24), following the High protocol, as all pellets weighed over 1 g. The non-separation elution method (Figure 2) was used in accordance with the manufacturer's instructions.

To evaluate performance, the binding bead and elution volumes were systematically varied. The Wait Elution Time was adjusted based on the volume of binding beads used. Please refer to Table 1 for the recommended wait times corresponding to different binding bead volumes.

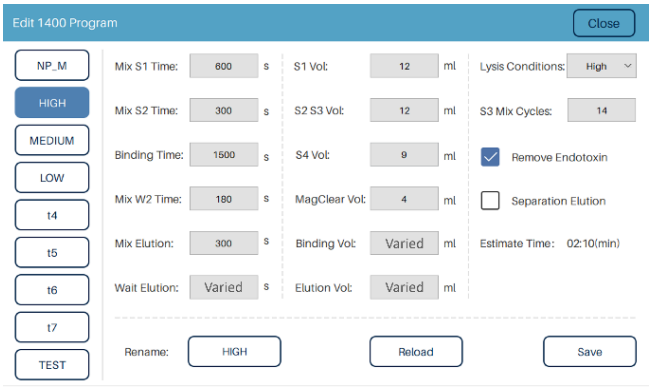

Figure 2. Program used in the AmMag™ Quatro 1400 to purify pcDNA3.1(+) plasmid. Protocol "High" was used for processing the pellets. The binding beads and elution volumes were systematically varied to study the effects. Wait elution time was adjusted according to the binding beads volume used.

Concentration, Purity, Quality, and Endotoxin Analysis

The concentration and purity of the purified pcDNA3.1(+) plasmid samples were determined with a NanoPhotometer® N50 (IMPLEN). Two µL of sample was loaded on the pedestal and the absorbance at 230, 260, and 280 nm was measured.

Endotoxin levels were measured using the Endosafe® nexgen-PTS™ (Charles River). Samples were diluted 1:100 mL/mL in LAL reagent water (GenScript) and loaded into Endosafe® LAL cartridges (Charles River).

Quality assurance was conducted using supercoil analysis and restriction enzyme digestion. Digestions were carried out with SmaI and/or EcoRI (New England Biolabs) according to the manufacturer's instructions. The resulting samples were loaded onto a 1.0% agarose gel, electrophoresed at 150 V for 20 minutes, and visualized under UV light. Supercoil analysis was performed using Image Lab software (Bio-Rad).

Results and Discussion

Analysis of Plasmid Yield with Binding Beads and Elution volume titration

To evaluate the impact of varying binding bead and elution volumes using the non-separation elution method on key parameters—including plasmid yield, concentration, and volume recovery—a series of conditions were tested. Binding bead volumes ranging from 1 mL to 3 mL and elution volumes from 0.38 mL to 2 mL were assessed across two biological replicates.

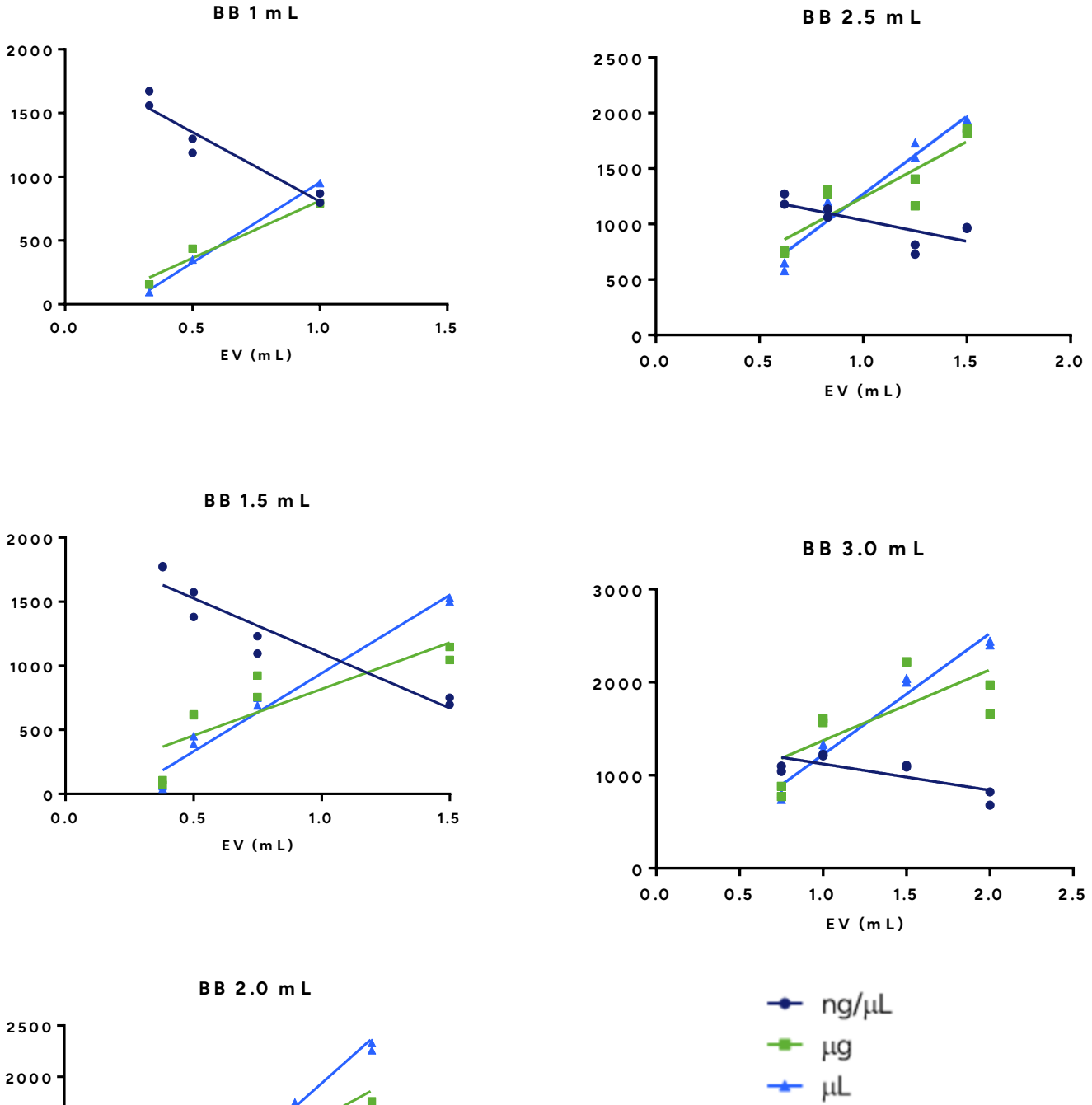

Figure 3. Binding beads and elution volume titration for high-copy plasmids in AmMag Quatro with Non-Separation Elution. Protocol "High" was used for processing the pellets. These scatter plots represent the correlation between plasmid yield (µg) and concentration (ng/µL) under different binding bead and final eluted sample volume (mL).

Results demonstrated a positive correlation between binding bead volume and plasmid yield (Figure 3), with yield increasing proportionally as the binding bead

volume increased. However, beyond 2.5 mL of binding beads, the yield plateaued—likely due to limitations in DNA availability. This suggests that increasing the bead volume beyond this point does not improve recovery.

It is not feasible to boost plasmid yield by increasing pellet weight beyond 1.5g, as the amount of lysis buffer in the prefilled reagent cartridge is fixed. Exceeding this capacity can result in incomplete lysis, ultimately leading to poor plasmid DNA yield and quality.

Increasing the elution volume, regardless of the binding bead volume used, resulted in higher plasmid yield due to more efficient elution of DNA from the magnetic beads. However, this also led to a decrease in plasmid concentration, as the DNA was diluted in a larger volume of elution buffer. Conversely, lower elution volumes produced higher concentrations of plasmid DNA, albeit with a slight reduction in total yield.

Compared to the yields obtained with separation elution [see App. Note Optimizing Plasmid Yield with the AmMag Quatro 1400 with Separation Elution], the non-separation elution shows significantly increased yields.

These results highlight the flexibility of the AmMag™ Quatro system, allowing users to adjust conditions to prioritize either yield or concentration based on specific experimental requirements.

Quality Analysis of the Purified Plasmids

To evaluate the impact of varying conditions using the non-separation elution method, multiple quality control (QC) metrics were assessed across different binding bead and elution volume combinations using high-pellet samples of a high-copy plasmid.

Purity measurements, including A260/280 and A260/230 ratios, ranged from 1.87 to 1.97 and 2.19 to 2.31, respectively—indicating minimal contamination from proteins or salts. Supercoiled plasmid content exceeded 90% in all samples, confirming high structural integrity under all tested conditions.

Agarose gel electrophoresis verified plasmid integrity (Figure 4A). Non-digested samples showed a predominant supercoiled conformation with no detectable genomic DNA contamination. Enzymatic digestion with EcoRI and SmaI produced bands of the expected sizes, further confirming the absence of structural anomalies. Densitometric analysis (Figure 4B) supported these findings, with all conditions showing greater than 90% supercoiled content. Additionally endotoxin levels remained below 0.1 EU/μg across all conditions tested (data not shown).

Together, these results demonstrate that the non-separation elution method yields high-quality plasmid preparations across a broad range of tested parameters, with consistent purity, structural integrity, and low endotoxin content.

Conclusion

The AmMag™ Quatro 1400 system offers users the flexibility to edit protocols in order to achieve desired plasmid concentration and yield. The use of non-separation elution significantly increases plasmid yield compared to the traditional separation elution method [see App. Note Optimizing Plasmid Yield with the AmMag Quatro 1400 with Separation Elution].

Additionally, the system allows users to select from different protocols based on specific yield and concentration requirements without compromising the quality of the purified plasmids.

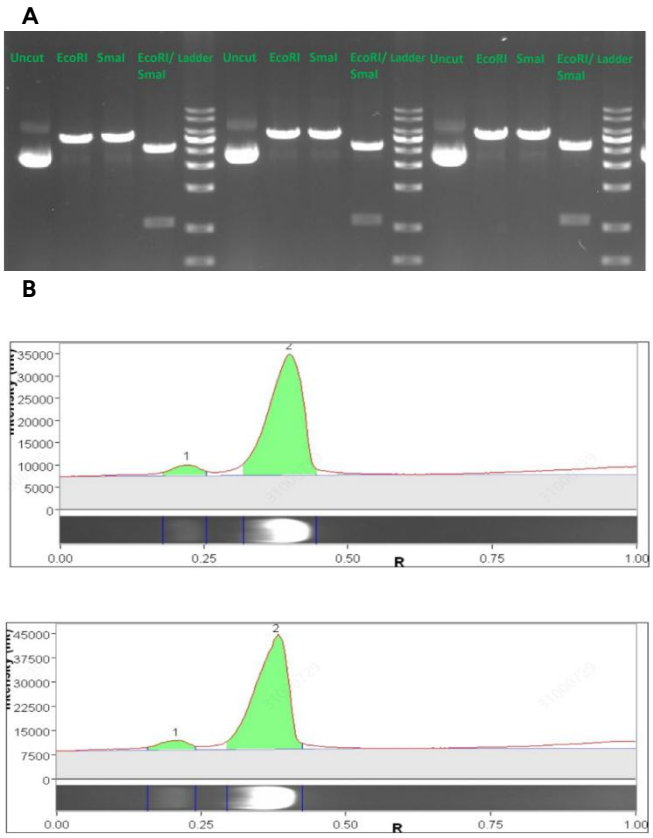

Figure 4. Quality analysis of the purified plasmids with AmMag™ Quatro. A) Agarose gel electrophoresis analysis of plasmid quality. Plasmid integrity was confirmed by Restriction Enzyme digestion with EcoRI and SmaI. B) Quantitative analysis of supercoil ratio was determined by densitometric analysis of the bands corresponding to the supercoiled and relaxed forms of plasmid DNA.

Summary

The AmMag™ Quatro 1400 system enables fully automated plasmid DNA purification while offering flexibility to optimize yield and concentration through different elution methods and adjustable binding bead and elution volumes. This adaptability supports a range of application needs without compromising plasmid quality.

Key findings include:

- When higher yields are required, the non-separation elution method can be used to significantly improve plasmid recovery.
- There is a trade-off between yield and concentration:
    - Higher elution volumes increase total yield but reduce plasmid concentration due to dilution.
    - Lower elution volumes yield more concentrated DNA, though with a slight reduction in total yield.
- The titration graph (Figure 3) serves as a useful guide for selecting the appropriate binding bead and elution volumes to achieve the desired balance of yield and concentration.
- Note: Increasing the pellet weight beyond 1.5g would results in lower yield and quality due to incomplete lysis

Tables

Binding beads volume (mL)	Wait elution time (s)
Up to 1.5	600
1.5-2.2	1200
2.2-3.0	1800

Table 1. Recommended Wait Elution Time for Different Binding Bead Volumes.
This table outlines the recommended wait time during the elution step for various binding bead volumes used in plasmid purification. Adhering to the correct wait time helps ensure that the final plasmid sample is free of residual ethanol, which could otherwise interfere with sensitive downstream applications. Using a shorter-than-recommended wait time may result in incomplete ethanol evaporation, leaving behind traces that could negatively impact results in applications such as transfection, IVT, or sequencing

Ordering Information

Instrument

Cat. No.	Product Name	Function
D00018	AmMag™ Quatro 1400 System Controller	Controller that can control up to 4 purification modules, simultaneously
D00019	AmMag™ Quatro 1400 Automation Purification Module	One module can purify up to 6 samples per run

Kits

Cat. No.	Product Name	Elution Buffer	Endotoxin Level (EU/μg)	Common Applications
L00882-24	AmMag™ Quatro Plasmid Purification Kit (Water)	Water	<0.10	General purpose applications, including those requiring transfection-grade plasmids
L00943-24	AmMag™ Quatro Plasmid Purification Kit (TE)	TE	<0.10	General purpose applications, including those requiring transfection-grade plasmids
L01031-24	AmMag™ Quatro Endofree Plasmid Purification Kit (Water)	Water	<0.01	General purpose and sensitive applications requiring plasmids with minimal endotoxin levels
L01122-24*	AmMag™ Quatro-AOF Plasmid Purification Kit (Water)	Water	<0.10	General purpose applications; suitable for GMP workflows
L01104-24*	AmMag™ Quatro-AOF Plasmid Purification Kit (TE)	TE	<0.10	General purpose applications; suitable for GMP workflows
L01124-24*	AmMag™ Quatro Endofree AOF Plasmid Purification Kit (Water)	Water	<0.01	General purpose applications; suitable for *In Vivo* and GMP workflows
L01144-24*	AmMag™ Quatro Endofree AOF Plasmid Purification Kit (TE)	TE	<0.01	General purpose applications; suitable for *In Vivo* and GMP workflows

* Available by custom order.