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AmMag^{TM} Quatro 1400 – Protocol Optimization Guide

Cat. No. D00018, D00019

The AmMag™ Quatro 1400 is a versatile and efficient system for plasmid DNA isolation. To ensure optimal performance and achieve the best plasmid yield and purity, it is crucial to fine-tune the protocol based on your specific sample conditions, such as pellet size, plasmid copy number, and desired yield.

This guide provides detailed steps for protocol optimization, focusing on key factors that impact plasmid recovery. It also includes troubleshooting solutions for common issues, helping you address challenges such as low yield, contamination, and inefficient elution. By following this guide, you can streamline your workflow and ensure reproducible, high-quality plasmid preparations.

1. Step-by-Step Protocol Optimization

a. Select the appropriate protocol based on pellet size:

- High: 1.0 to 1.5 g
- Medium: 0.8 to 1.0 g
- Low: 0.6 to 0.8 g

b. Adjust binding volume according to plasmid copy number and yield:

- Low Copy/Low Yield: 0.3 to 0.6 mL
- Medium Copy/Medium Yield: 0.7 to 1.0 mL
- High Copy/High Yield: 1.0 to 1.7 mL

c. Set the elution volume relative to the binding volume:

- **General Rule:** To achieve higher plasmid concentration, use a smaller elution volume. However, the elution volume should not be less than 60% of the binding volume.

d. Optimize Yield by Adjusting Elution Settings:

• To improve plasmid yield, consider disabling the **“Separation Elution”** option — but only after confirming that your current protocol consistently yields **500–1000 ng/µL** of plasmid.

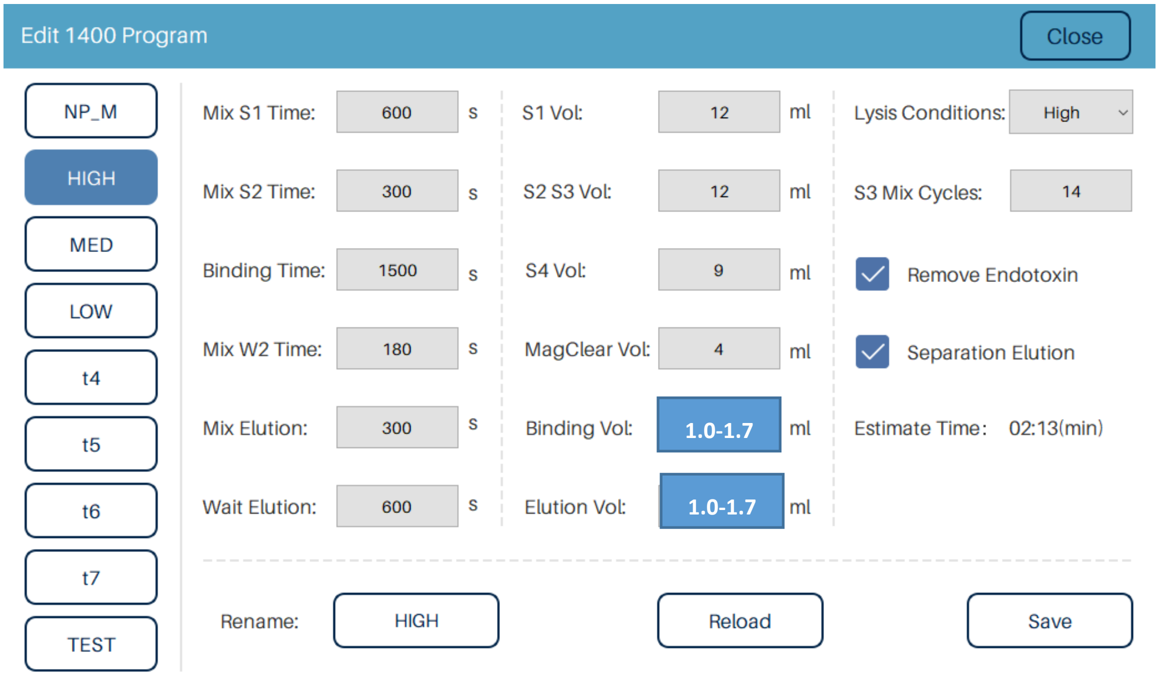
Disabling “Separation Elution” enables a **non-separation elution** method, which can significantly enhance yield by minimizing volume loss.

e. **Check or uncheck the endotoxin removal step**.

When the endotoxin removal step is unchecked, the endotoxin levels will be higher in the final samples than what GenScript promises. However, this will shorten the total time taken for the purification if the endotoxin levels are not a concern.

2. Protocols based on pellet weight

- **Program "High"**
  - **Pellet Size:** 1.0 to 1.5 g
  - **Plasmid Type:** High copy/High yield
  - **Typical Yield:** 0.5 to 1.0 mg



Edit 1400 Program

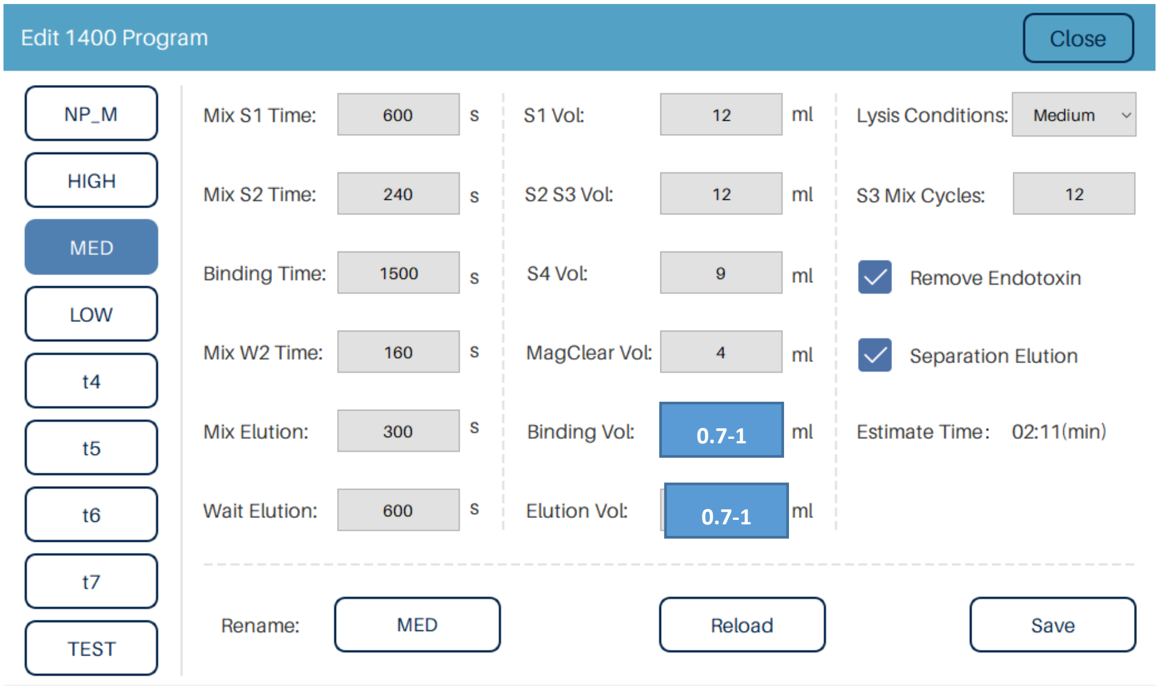
Mix S1 Time: 600 s
Mix S2 Time: 300 s
Binding Time: 1500 s
Mix W2 Time: 180 s
Mix Elution: 300 s
Wait Elution: 600 s

S1 Vol: 12 ml
S2 S3 Vol: 12 ml
S4 Vol: 9 ml
MagClear Vol: 4 ml
Binding Vol: 1.0-1.7 ml
Elution Vol: 1.0-1.7 ml

Lysis Conditions: High
S3 Mix Cycles: 14
☑ Remove Endotoxin
☑ Separation Elution
Estimate Time: 02:13(min)

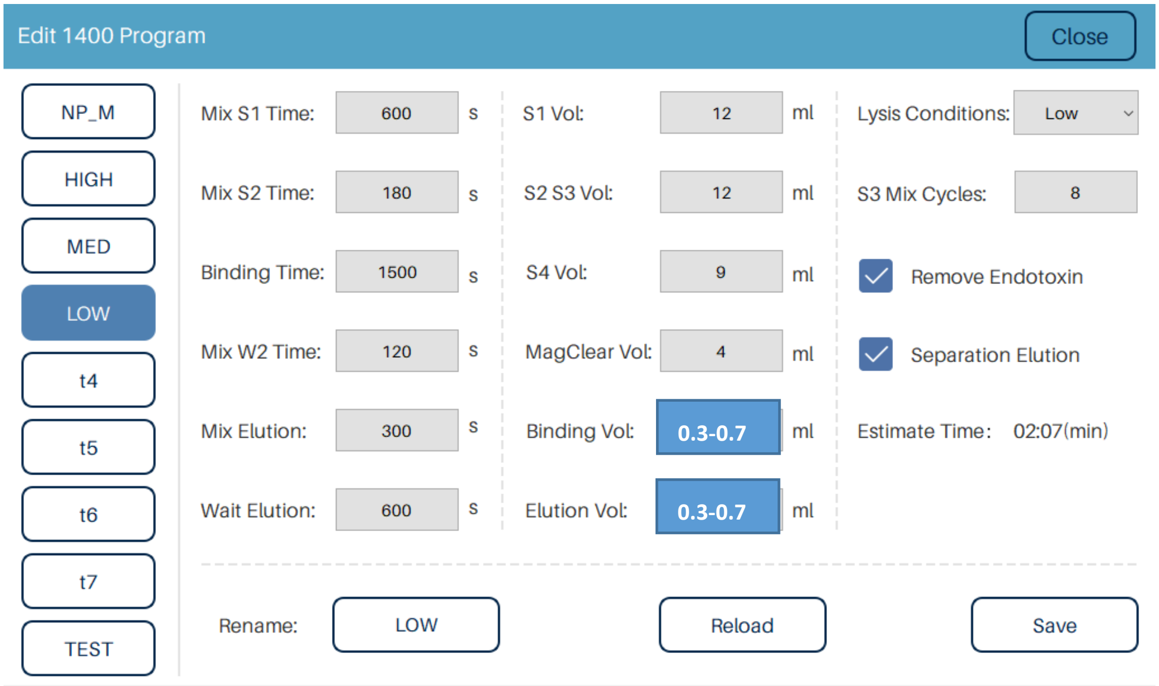
Rename: HIGH

- **Program "Medium"**
  - **Pellet Size:** 0.8 to 1.0 g
  - **Plasmid Type:** High copy/High yield
  - **Typical Yield:** 0.3 to 0.5 mg



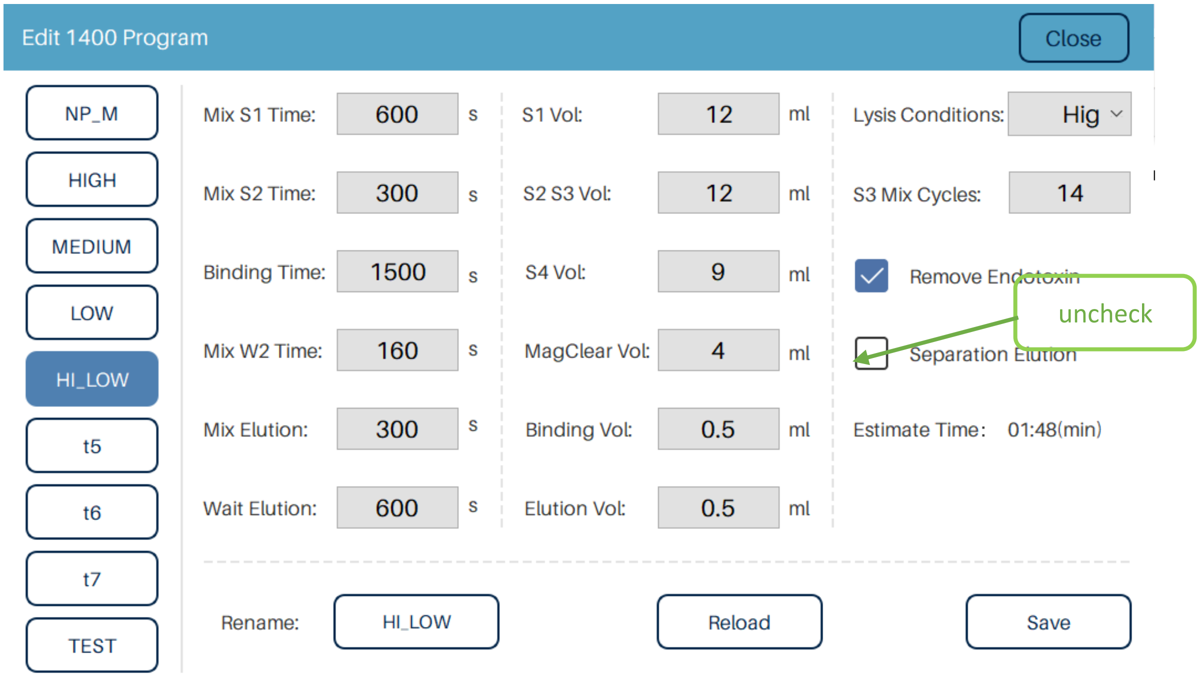
Mix S1 Time: 600 s
Mix S2 Time: 240 s
Binding Time: 1500 s
Mix W2 Time: 160 s
Mix Elution: 300 s
Wait Elution: 600 s
S1 Vol: 12 ml
S2 S3 Vol: 12 ml
S4 Vol: 9 ml
MagClear Vol: 4 ml
Binding Vol: 0.7-1 ml
Elution Vol: 0.7-1 ml
Lysis Conditions: Medium
S3 Mix Cycles: 12
☑ Remove Endotoxin
☑ Separation Elution
Estimate Time: 02:11(min)
Rename: MED

- **Program “Low”**
    - **Pellet Size:** 0.3 to 0.8 g
    - **Plasmid Type:** High copy/High yield
    - **Typical Yield:** 0.1 to 0.3 mg



Mix S1 Time: 600 s
Mix S2 Time: 180 s
Binding Time: 1500 s
Mix W2 Time: 120 s
Mix Elution: 300 s
Wait Elution: 600 s
S1 Vol: 12 ml
S2 S3 Vol: 12 ml
S4 Vol: 9 ml
MagClear Vol: 4 ml
Binding Vol: 0.3-0.7 ml
Elution Vol: 0.3-0.7 ml
Lysis Conditions: Low
S3 Mix Cycles: 8
☑ Remove Endotoxin
☑ Separation Elution
Estimate Time: 02:07(min)
Rename: LOW

- **Program “High_Low”**
    - **Pellet Size:** 1.0 to 1.5 g
    - **Plasmid Type:** Low copy/Low yield
    - **Typical Yield:** 0.1 to 0.6 mg



Mix S1 Time: 600 s
Mix S2 Time: 300 s
Binding Time: 1500 s
Mix W2 Time: 160 s
Mix Elution: 300 s
Wait Elution: 600 s

S1 Vol: 12 ml
S2 S3 Vol: 12 ml
S4 Vol: 9 ml
MagClear Vol: 4 ml
Binding Vol: 0.5 ml
Elution Vol: 0.5 ml

Lysis Conditions: Hig
S3 Mix Cycles: 14
☑ Remove Endotoxin
☐ Separation Elution
Estimate Time: 01:48(min)

Rename: HI_LOW

NOTE:

When larger binding beads volumes are selected, the wait elution time (air drying time) must be increased to prevent ethanol residue in the final sample, which may interfere with downstream applications (e.g., restriction enzyme digestion or transfection).

Binding Beads Volume	**Wait elution time**
Up to 1.5 mL	600 s
1.5-2.2 mL	1200 s
2.2-3.0 mL	1800 s

3. Troubleshooting

Issue Description	Proposed Solution
Low yield	Check binding and elution volumes, ensuring the appropriate protocol settings are used. Set binding time parameter from 1500 – 1800s.
Low 260/280	This is an uncommon issue. This issue may indicate protein contamination in the sample caused by lysis failure and consequently the entire prep will fail. Review the protocol settings and adjust based on the pellet weight and plasmid expected yield.
Low 260/230	When the plasmid concentration is very low (less than 200 ng/ul) the 260/230 ratio will be impacted due to low 260nm reading. We suggest eluting in smaller volume to make your plasmid DNA more concentrated. Non-separation elution method can also lower the 260/230 ratio.
High Salt Concentration	The standard protocols for high pellets use 180s of mixing time for W2 washing buffer – "Mix W2 time". To minimize salt carryover increase "Mix W2 time" from 180 to 240s.
Inefficient Elution	The elution volume is recommended to be equal to the binding volume for high elution efficiency. If using a smaller elution volume to achieve higher plasmid concentration, the user should ensure it is at least 60% of the binding beads volume. Otherwise, this will cause poor elution efficiency.
gDNA Contamination	To reduce gDNA contamination, work with pellets up to 1g (ideal between 0.8 and 1g). Using the medium protocol, reduce the S3 Mix cycles from 12 to 6 gradually by decrements of 2. In addition, reduce "Mix S2 time" from 240 to 180s by decrements of 40s. The shearing force of the tips may lead to some gDNA contamination. Note: you can only change the parameters if the clarified lysate is very smooth,

Issue Description	Proposed Solution
	otherwise it could cause incomplete lysis.
Incomplete Pellet resuspension or Frozen Samples	Increase Mix S1 time to 600-800s or add up to 1ml of PBS or nuclease free water and vortex the pellet prior to loading the sample tube into the instrument.
High Ethanol Content	For non-separation elution, you can adjust the "wait elution time" (which corresponds to the air-drying time) based on the volume of the binding beads is used, otherwise you may observe high ethanol contents in your samples. • up to 1.5ml binding beads – set "wait elution" to 600s • 1.5 – 2.2 ml binding beads – set "wait elution" to 1200s • 2.2 – 3 ml binding beads – set "wait elution" to 1800 s
Low Supercoil	Several factors influence plasmid supercoiling, including the activity of topoisomerases, plasmid size, and DNA sequence characteristics. Inadequate handling or suboptimal storage conditions, such as prolonged exposure to water, can lead to plasmid degradation and loss of supercoiling. To preserve supercoiling, it is recommended to store plasmids in TE buffer rather than water. The AmMag™ Quatro protocols offer numerous adjustable parameters to optimize plasmid yield and purity, including supercoiling.
Plasmid Yields Lower than Expected	Run a side-by-side comparison with a manual kit to rule out the possibility of issues during plasmid culture growth.
AAV plasmids low yield	Refer to the growth conditions and protocol optimization for AAV plasmids.

This guide should help in optimizing the AmMag™ Quatro 1400 protocols to achieve desired plasmid yields and purity. By following the recommended adjustments and troubleshooting steps, you can fine-tune the protocol for consistent and reliable results.

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