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Pellet Preparation Protocol for AmMag™ Quatro 1400 Maxi-Scale Plasmid Purification

Cat. No. D00018, D00019

I. Introduction

The AmMag™ Quatro 1400 system is designed for Maxi-Scale automated plasmid DNA purification from bacterial cultures.

This protocol outlines the recommended procedure for preparing bacterial pellets for maxi-scale plasmid purification using the Quatro 1400. Ensuring optimal pellet mass and consistent culture conditions is critical for maximizing plasmid yield and quality to achieve reproducible results and streamline your plasmid purification workflow.

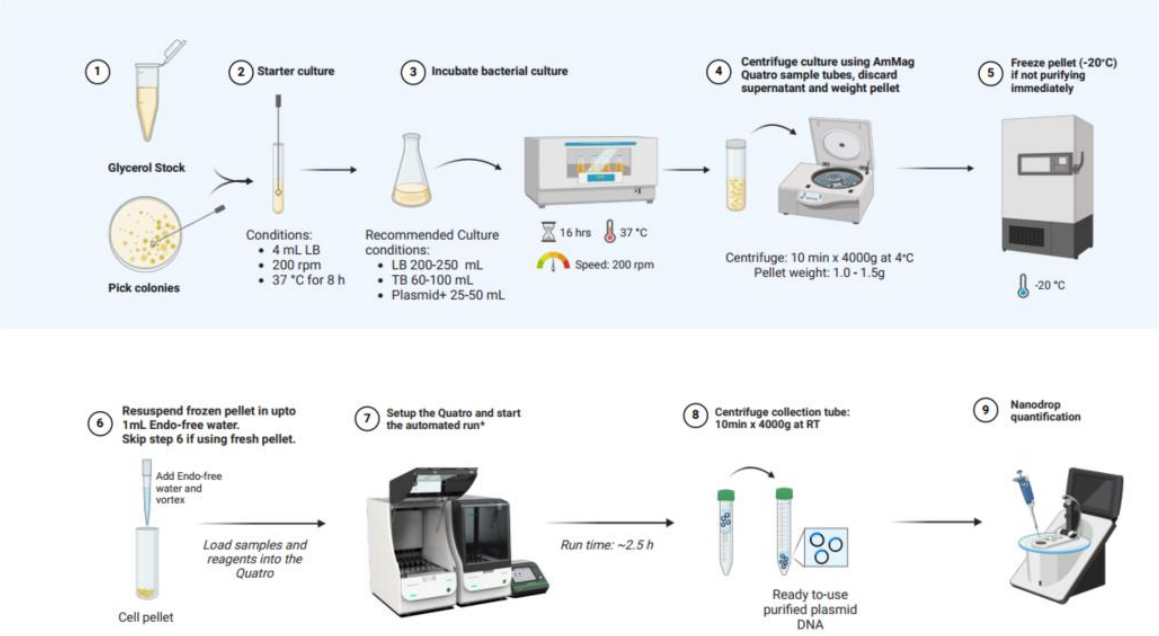

FIGURE 1 AmMag™ Quatro Maxi 1400 Purification Process

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II. Protocol

1. Starter Culture Preparation

1) Pick a single colony from a freshly streaked selective plate to inoculate 2–4 mL of starter culture in a 15 mL tube.
    - Optional: inoculate 5µl from glycerol stock.
2) Incubate the starter culture in a shaker at 37°C and ~200 rpm for 8 h, or until the OD_{600} reaches 2.5.
    - If OD_{600} has not reached 2.5, incubate for at least an additional 45 minutes.

2. Large-Scale Culture Growth

3) Add 1:1000 dilution of the starter culture to fresh media (LB, 2xYT, TB or Plasmid+) along with the appropriate selective antibiotic.
    - Use a flask with a volume at least 4 times the culture volume.
    - Check culture media recipe and volume recommendation for achieving optimal pellet weight (1.0 – 1.5 g) in the next protocol section.
4) Incubate the culture in a shaker at 37°C and ~200 rpm for ~16 h.

3. Harvesting the Bacterial Pellet

5) Harvest the culture after ~16 hours of growth[1].
6) Pre-weigh each empty Quatro Sample Collection Tube and record the weight.
7) Transfer the culture into the pre-weighed tubes[2].
8) Centrifuge at 4°C, 4000 x g for 10 min. Discard the supernatant.
9) Weigh the tube containing the pellet and subtract the empty tube weight to determine pellet mass.
    - The pellet should range between 1.0 – 1.5 g.
    - If necessary, add up to 50 mL of bacterial culture to the same tube and repeat centrifugation to achieve the desired pellet weight. [3]

4. Pellet Storage and Preparation for Use

10) For immediate use: Load the pellets directly into the AmMag™ Quatro 1400 and start plasmid purification run immediately.
11) For future use: Store the pellets at -20°C.
12) If using frozen pellets: Add 0.5–1.0 mL of endonuclease-free water and vortex until fully resuspended.
    - Do not exceed 15 minutes from thawing to starting the purification process.

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III. Notes & Recommendations

[1] This time range is the transition from logarithmic into stationary growth phase and it is recommended to not let culture overage into later stationary phase to avoid DNA degradation and excess of endotoxin in the final sample.

[2] To assure the most accurate pellet weight, we recommend to pre-weight each empty Quatro sample collection tube as there is small mass variation for each tube. The wet pellet weight is only required when a new growth condition is being tested to ensure that the pellet size is in the recommended range of 1-1.5 g.

[3] Culture media type volume recommendation for achieving optimal pellet weight (1.0 – 1.5 g).

Media	Culture volume	Pellet weight
LB media	200 – 250 mL	1 to 1.5g
2xYT	100 – 150 mL	
TB media	60 – 100 mL	
Plasmid+ media	25 – 50 mL	

[4] It is recommended to use Quatro Sample Collection Tubes to centrifuge the bacterial cultures as transferring pellets to these tubes afterwards may cause reduction of biomass, increase chances of contamination and degradation.

[5] For Plasmid+ media, it is strongly recommended to use large, baffled flasks to ensure sufficient aeration relative to the culture volume (see Table below). Proper aeration is critical for high-efficiency plasmid production. Cultures should be adjusted to the recommended volumes, and shaking speed should be maintained above 350 rpm to promote optimal oxygen transfer.

Additionally, Escherichia coli DH5α is the preferred host strain for plasmid DNA production

Prep type	Recommended Volume	Recommended Vessel
MAXI Prep	35-50mL	125-200 mL baffled Flask

IV. Appendix A - Culture Media Recipes

Consistent media preparation is crucial for reproducible culture growth and downstream plasmid application. The recipes for commonly used bacterial growth media (LB, 2xYT, TB and Plasmid+ media) are provided in this section.

1. **Luria-Bertani (LB) Medium**
   - Tryptone: 10 g
   - NaCl: 10 g
   - Yeast extract: 5 g
   - MilliQ water to 950 mL

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- Adjust pH to 7.0 with ~0.2 mL of 5N NaOH
- Adjust final volume to 1 L with MilliQ water
- Autoclave

2. 2X YT Medium

- Tryptone: 16 g
- Yeast extract: 10 g
- NaCl: 5 g
- MilliQ water to 900 mL
- Adjust pH to 7.0 with 5N NaOH
- Adjust final volume to 1 L with MilliQ water
- Autoclave

3. Terrific Broth (TB) Medium

TB-A:

- Yeast extract: 24 g
- Tryptone: 12 g
- Glycerol: 4 g
- MilliQ water to 900 mL
- Autoclave

TB-B:

- KH_2PO_4: 2.3 g
- K_2HPO_4: 16.4 g
- MilliQ water to 100 mL
- Autoclave

Final TB Medium Preparation: Mix 900 mL of TB-A with 100 mL of TB-B before use.

4. Plasmid+ Medium

- A commercially available rich media from Thomson Instruments.

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Manufacturer: Nanjing GenScript Biotech Co., Ltd. No. 28 Yongxi Road, Jiangning District, Nanjing, Jiangsu, China

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854 1-732-885-9188