

Protocol:

Pellet Preparation Protocol for Plasmid Purification Using the AmMag™ Quatro Maxi-1400 and Thomson Ultra Yield® Plasmid System

Description

This protocol provides a step-by-step guide for preparing bacterial pellets using the AmMag™ Quatro Maxi-1400 and Thomson Plasmid+® Enriched Media with Ultra Yield® Flasks, ensuring consistent, high-quality plasmid DNA for use in sensitive downstream applications.

Method

Transformation

- 1) Perform the transformation of the plasmid into the cell line of choice following the manufacturer's recommended protocol.
 - For best plasmid purification results, freshly transformed plates are strongly recommended.

Starter Culture

- 2) Inoculate 4 mL of Plasmid+® Media (Thomson; Part No. 446300) in a 15 mL test tube with a single colony picked from a freshly transformed plate.
- 3) Grow the starter culture at 37°C with shaking at 220 RPM for 8 hours or until the OD600 reaches 2.5.
 - If the OD600 does not reach 2.5, continue shaking in increments of 45 minutes until the target OD600 is reached.

Large-Scale Culture

- 4) Add 30 mL of Plasmid+® Media into a 125 mL Ultra Yield® Flask (Thomson; Part No. 931147).
- 5) Add Antifoam 204 (Sigma-Aldrich; Cat. No. A8311-50ML) at a 1:5000 dilution into the flask.
 - Add 6 µL of Antifoam 204 per 30 mL of Plasmid+® Media.
- 6) Add the antibiotic of choice at the preferred concentration into the flask.
- 7) Inoculate the media by adding the starter culture at 1:1000 dilution into the flask.
 - Add 30 µL of starter culture per 30 mL of Plasmid+® Media.
- 8) If multiple flasks of the same bacterial culture are required, prepare a master mix of steps 4–7 and distribute in 30 mL increments into each 125 mL flask.
- 9) Repeat steps 4–7 as needed for different bacterial cells.
- 10) Secure the flask opening with a 125 mL Ultra Yield® Vent Cap (Thomson; Part No. 899109).
- 11) Incubate the culture at 37°C with shaking at 350 RPM for 16 hours in a 25 mm orbital shaker.

Harvesting Bacterial Pellet

- 12) Pre-chill centrifuge to 4°C.
- 13) Pre-weigh and record each empty 50mL Quatro Sample Collection Tube needed for bacterial pellet.
 - Label tube as needed.
- 14) After 16h cultivation, transfer the 30mL culture into an empty Sample Collection Tube.
- 15) Centrifuge tube at 4000 x g in 4°C for 10min.
- 16) Discard vent cap.
- 17) 125mL Ultra Yield® flasks could be sterilized and reused up to 3 times.
- 18) After centrifugation, discard the media.
- 19) Weigh the tube containing the pellet and from it, subtract the weight of the empty tube.
 - With optimal culture conditions, bacterial pellet should be between 1.30 to 1.50g.

Pellet Storage and Preparation for Use

- 20) For immediate use:
 - Load the pellet directly into the AmMag™ Quatro Maxi-1400 and start plasmid purification run immediately.
 - For more information regarding protocols, please visit "Guides on Protocol Settings for Plasmid Purification Based on Sample Input" on our website: [AmMag™ Quatro Maxi Automated Maxi-Plasmid Purification](#)
- 21) For future use:
 - Store bacterial pellets in -20°C.
 - When needed for purification, resuspend and vortex the bacterial pellet in up to 1mL of nuclease-free water or 1X PBS and thaw in room temperature for up to 15min.