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AmMag™ Quatro Plasmid Mini kit

Instruction Manual

Cat. No. L01037-48

Cat. No. D00056

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The operator must carefully read the datasheet before using this product.

This product is for research use only, not for clinical diagnosis.

1. Product Description

The AmMag™ Quatro 1100 automated purification system is designed to efficiently purify plasmid DNA from Escherichia coli (E. coli) using the AmMag™ Quatro Plasmid Mini kits. The complete system (Cat. No. D00055) includes the system controller, purification modules, and the appropriate plasmid purification kits. Each AmMag™ Quatro 1100 Automation Purification Module (Cat. No. D00043) can process 48 samples per module, and the system controller can manage up to four independent modules simultaneously, allowing for the purification of up to 192 samples in a single run. Each module provides fully automated, high-yield plasmid purification with reliable purity.

The AmMag™ Quatro Low-Endotoxin Plasmid Purification Mini Kit (Cat. No. L01037-48) is specifically designed for optimal endotoxin removal. The kit utilizes GenScript's exclusive endotoxin-removal technology to produce high-quality plasmid DNA with minimal endotoxin levels.

Table 1 AmMag™ Quatro 1100 automated purification system

| Cat. No. | Product Name | Specifications |
|----------|---|----------------|
| D00055 | AmMag™ Quatro 1100 Mini Controller | 1 unit |
| D00043 | AmMag™ Quatro 1100 Automation Purification Module | 1 unit |

For detailed operation instructions, please refer to the *AmMag™ Quatro 1100 automated small -scale magnetic beads purification system Manual*.

2. Kits and Supplemental Components

| Cat. No. | Product Name | Specifications | Components | Volume/Quantity |
|-----------|---|----------------|----------------------------|-----------------|
| L01037-48 | AmMag™ Quatro Low-Endotoxin Plasmid Purification Mini Kit, 48 Preps | 48 samples | Buffer S1 | 21 mL |
| | | | Buffer S2 | 35 mL |
| | | | Buffer S3 | 35 mL |
| | | | Buffer BD | 42 mL |
| | | | Buffer EQ1 | 14 mL |
| | | | Buffer EQ2 | 8 mL |
| | | | Buffer ER | 106 mL |
| | | | Buffer WB1 ^a | 16 mL |
| | | | Buffer WB2 ^b | 30 mL |
| D00056 | AmMag™ Quatro Plasmid Mini Consumables, 96 Preps | 96 samples | 48-Deep Well Plates | 2 plates |
| | | | 4-Column Reagent Reservoir | 6 plates |
| | | | 1000 µL Tips | 10 boxes |
| | | | Quatro Reaction Plates | 6 plates |
| | | | 96-Deep Well Plates | 2 plates |

- a. Please add 38 mL of anhydrous ethanol (200 Proof) to the bottle of WB1 before use.
- b. Please add 70 mL of anhydrous ethanol (200 Proof) to the bottle of WB2 before use.

Reagents and Consumables Provided by the Scientist (Not Included in the Kit):

| Reagent/Consumable | Quantity | Note |
|------------------------------|----------------------|---|
| Anhydrous ethanol (200Proof) | 110 mL (approximate) | Recommendation: freshly mix ethanol with buffers according to instructions in section 5.2 prior to purification. |
| Plate seal(s) | 1-2 per sample plate | Used for sample storage |

3. Storage Conditions

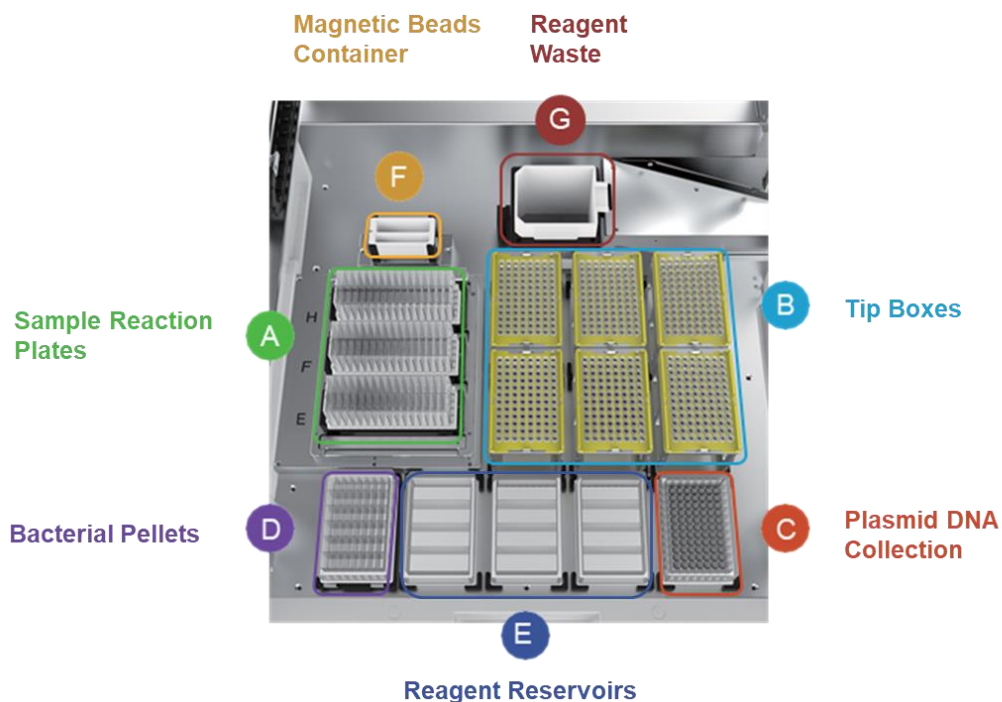
The kit should be stored at room temperature (15-25°C).

Note:

Reagents may form precipitates if stored at temperatures below 15°C. To dissolve the precipitate, store the bottles at room temperature for 24 hours or heat them in a 37°C water bath for 15 minutes. Shake the bottles thoroughly afterward.

4. AmMag™ Quatro 1100 Deck

Visual Layout of Labware Positions on AmMag™ Quatro 1100 Deck



Note:

Before operating the AmMag™ Quatro 1100 instrument and its corresponding consumables, users must thoroughly read the *AmMag™ Quatro 1100 automated small-scale magnetic beads purification system Manual*. For the initial use, please add 38 mL of anhydrous ethanol into the bottle of WB1 and 70 mL of anhydrous ethanol into the bottle of WB2, and thoroughly mix the solution.

5. Instructions for Use

Given the complexity of samples and the diversity of experimental conditions, the following instructions can serve as a reference. It is advisable to conduct a pre-experiment to achieve the optimized experimental conditions.

Note:

The yield and quality of purified plasmid DNA are highly dependent on bacterial culture conditions, the types of bacteria, plasmid copies and various other factors. Users will need to optimize their experimental setup to achieve the best results.

5.1 Prepare and Setup the Bacterial Samples

This protocol is designed for the purification of plasmid DNA from bacterial cultures. The required bacterial cell mass for optimal plasmid extraction is determined by the optical density at 600 nm (OD₆₀₀) and the culture volume. The ODV is calculated using the formula:

$$\text{ODV} = \text{OD}_{600} \times \text{Culture Volume (mL)}$$

For effective plasmid purification, a ODV between 10 and 15 is recommended. If purifying a larger ODV (cell mass), it may be necessary to adjust both the cell lysis conditions and the purification parameters in the software program accordingly.

Bacterial cultures may be grown in either culture tubes or 48-deep well plates, as described below:

5.1.1 Growing Cultures in Tubes

1. **Inoculation:** Pick a single isolated colony from a freshly streaked selective agar plate and inoculate it into 3-5 mL of medium supplemented with the appropriate selective antibiotic. Incubate the culture overnight at 37°C, with shaking at 250-300 rpm.
2. **Measurement of OD₆₀₀:** Measure the OD₆₀₀ of the overnight culture to assess cell density. Transfer an appropriate volume of culture to a 48-deep well plate, ensuring the target ODV (10–15) is achieved.
3. **Centrifugation:** Centrifuge the 48-deep well plate at 4000 × g for 10 minutes at 4°C to pellet the bacterial cells.
4. **Loading:** After centrifugation, place the 48-deep well plate in the designated area (Figure 1) and proceed to the next step (5.2).

5.1.2 Growing Cultures Directly in 48-Deep Well Plates

1. **Inoculation:** Add 1-2.5 mL of medium containing the selective antibiotic to each well of a 48-deep well plate.

2. **Colony Picking:** Pick a single isolated colony from a freshly streaked selective agar plate and inoculate each well using a sterile 10-20 μL pipette tip. The tip can either be left inside the well or removed after performing a gentle resuspension (up-and-down motion) to ensure proper inoculation.
3. **Sealing and Aeration:** Seal the plate with a plate cover and create a small hole in each well's seal to allow for adequate aeration during incubation.
4. **Incubation:** Incubate the 48-deep well plate overnight at 37°C with shaking at 800 rpm to promote bacterial growth.
5. **Centrifugation:** Centrifuge the plate at 4000 \times g for 10 minutes at 4°C to pellet the bacterial cells.
6. **Loading:** After centrifugation, place the 48-deep well plate in the designated area (Figure 1) and proceed to the next step (5.2).

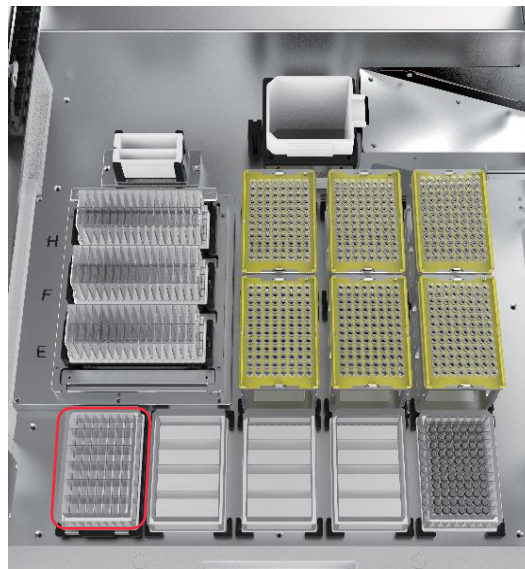


Figure 1. Location of bacterial pellets

5.2 Preparing the Reagents

- 1) Add the anhydrous ethanol to the WB1 and WB2 bottles using the following volumes followed by thoroughly mix the solutions.

| WB Buffer | Anhydrous ethanol (200 Proof) |
|-------------|-------------------------------|
| WB1 (16 mL) | 38 mL |
| WB2 (30 mL) | 70 ml |

- 2) Dispense each reagent into the designated section of the 4-Column Reagent Reservoir (Figure 2).

3) Place the Reagent Reservoirs (x3) in the specified location on the AmMag™ Quatro 1100 deck (as shown in Figure 3).

| | | |
|----|-----|----|
| S1 | WB1 | ER |
| S2 | WB2 | ER |
| S3 | WB2 | - |
| BD | EB | - |

Figure 2. Location of each reagent in the 4-Column Reagent Reservoirs

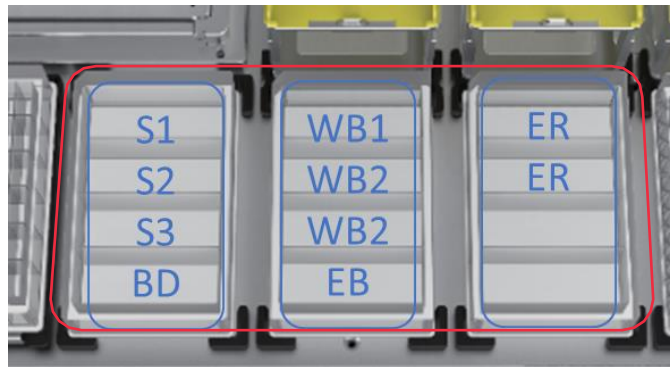


Figure 3. Location each reagent in the 4-Column Reagent Reservoirs

NOTE:

- Ensure that each reagent is placed in the correct position and orientation on the AmMag™ Quatro 1100 deck (Refer to Figure 4).
- The 4-Column Reagent Reservoirs can be reused. Wash the reservoirs thoroughly after each purification with Milli-Q water.

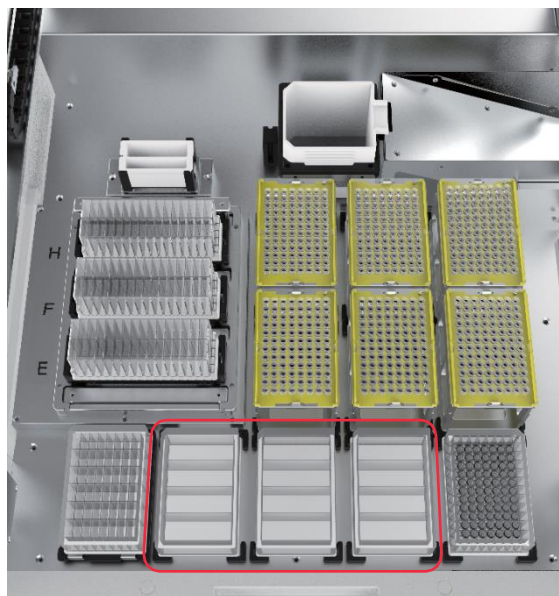


Figure 4. Location of each reagent

If endotoxin control is required, soak the reservoirs in **0.1 M NaOH** for **4 hours** after use, and then rinse with Milli-Q water.

5.3 Setting up the Purified Plasmid DNA Collection Plate

Place the 96-Deep Well Plates (Figure 5) in the designated area on the AmMag™ Quatro 1100 deck for the final step of purified plasmid DNA collection.

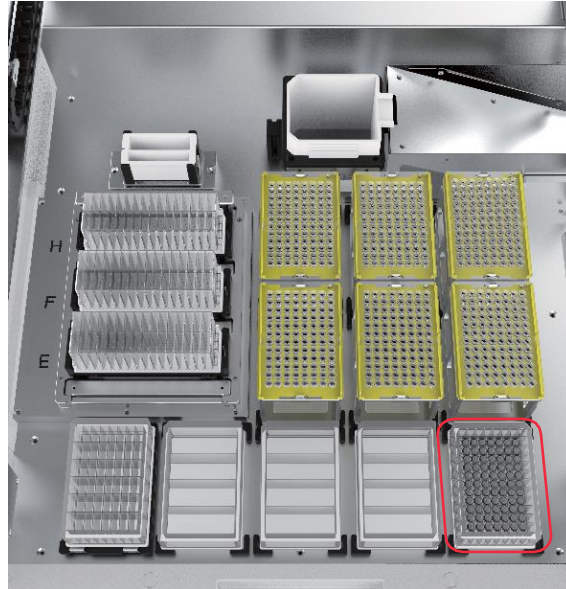


Figure 5. Location of plasmid DNA collection plate

5.4 Setup of the Reaction Plates

Place the AmMag™ Quatro Reaction Plates (Figure 6 & 7) correctly in the designated area of AmMag™ Quatro 1100 deck.

NOTE:

- a. Each AmMag™ Quatro Reaction Plate can be used to purify up to 16 samples at once. To process 48 samples, you will need 3 sets of Reaction Plates.

b. The AmMag™ Quatro Reaction Plates are available for purchase separately from GenScript (Cat. No. D00050).

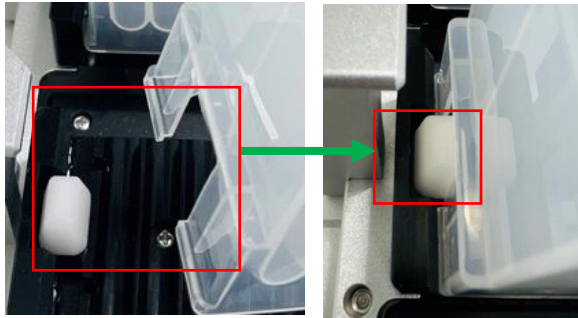


Figure 6. Set the 16-Sample Reaction Plates

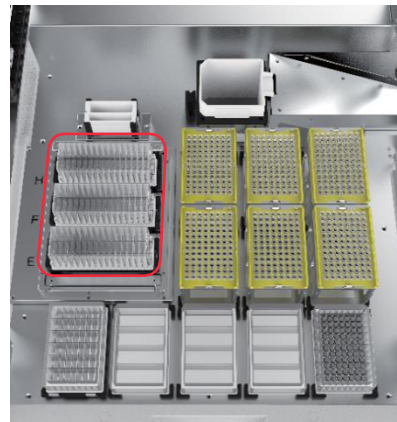


Figure 7. Location of 16-Sample Reaction

5.5 Setup the Tip Box

Load 96-Tip to the Rack in the correct orientation (Figure 8), then place the Tip Boxes (Figure 9) in the designated area of AmMag™ Quatro 1100 deck.

NOTE:

The 1000 μ L Tip Box must be loaded with the correct orientation. A maximum of six 96-Tip boxes can be placed in the designated area.

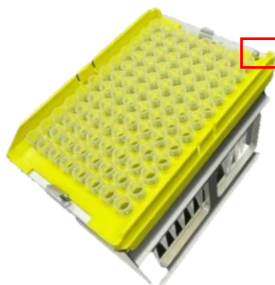


Figure 8. The 1000 μ L Tip Box with 96 Tips

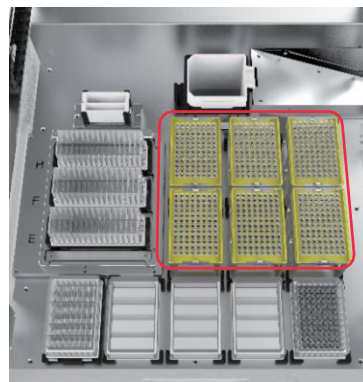


Figure 9. Location of Tips Box

5.6 Setup of the EQ Solutions

Transfer EQ1 and EQ2 from the bottles to the designated section in AmMag™ Quatro Magnetic Beads Reservoir and place the Reservoir (Figure 10) on the AmMag™ Quatro 1100 deck.

NOTE:

a. Ensure that EQ1 and EQ2 are correctly positioned on the AmMag™ Quatro 1100 deck.

b. The AmMag™ Quatro Magnetic Beads Reservoir can be reused. It is recommended to wash it with Milli-Q water after each purification.

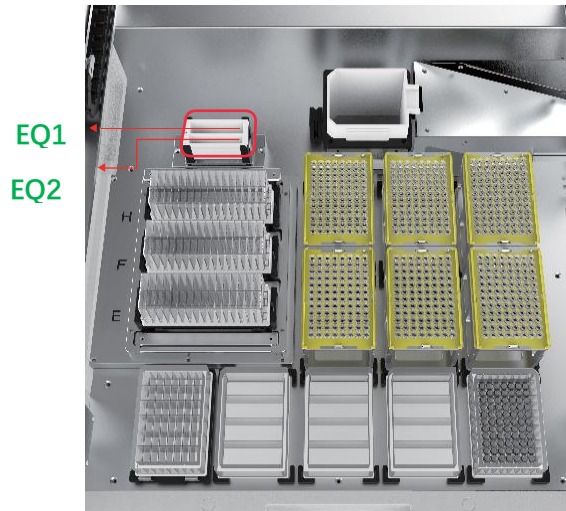


Figure 10. Location of Magnetic Beads

5.7 Set the Waste Container

Place the AmMag™ Quatro Waste Container (Figure 11) in the designated area of AmMag™ Quatro 1100 deck for waste collection. The Waste Container can be reused. It is recommended to dispose of the waste and clean the container after each purification process.

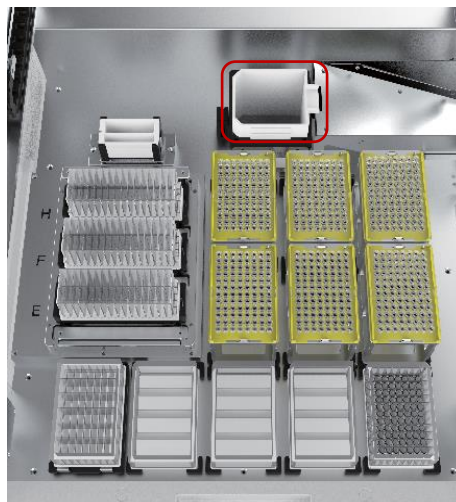


Figure 11. Location of Waste Container

5.8 Starting the Purification Program

Once the instrument is set up correctly, users can start the automated program according to the operation manual of *AmMag™ Quatro 1100 automated small scale magnetic beads purification system*.

6. Related Products

| Cat. No. | Product Name | Specifications |
|----------|-------------------------------|----------------|
| D00050 | AmMag™ Quatro Reaction Plates | 24 pcs/Box |

For research use only. Not intended for human or animal clinical trials, therapeutic or diagnostic use.

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