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Aurum™ Plasmid Mini Kit

Instruction Manual

For technical service, call your local Bio-Rad office, or
in the US, call 1-800-424-6723

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Section 1

Introduction

The new Aurum plasmid mini kit is optimized for the purification of up to 20 µg of plasmid DNA, rapidly and inexpensively, without the use of toxic reagents or alcohol precipitations. The use of membranes to bind and purify plasmid DNA, in combination with an optimized column design, minimizes handling and allows plasmid purification to be carried out in either a vacuum or spin format. In either format, the final elution is carried out in a microcentrifuge. Plasmid DNA purified with the Aurum plasmid mini kit is eluted into a small volume of aqueous buffer and is free of salts, bacterial chromosomal DNA, and RNA. The exceptional purity of the plasmid produced by this system makes it ideal for use in automated fluorescent sequencing and in any other molecular biology application.

Section 2

Kit Components

The Aurum plasmid mini kit contains the following components:

| | |
|-------------------------|------------------------|
| resuspension solution | 25 ml |
| lysis solution | 25 ml |
| neutralization solution | 40 ml |
| wash solution | 25 ml (5x concentrate) |
| elution solution | 16 ml |
| plasmid mini columns | 100 |
| 2 ml capless wash tubes | 100 |

The solutions are specifically formulated for the Aurum plasmid mini kit. They are NOT interchangeable with solutions used in other kits or protocols.

Section 3

Storage Conditions

Solutions and columns should be stored at room temperature. If precipitation is observed in any solution, warm solution to 37°C to redissolve, and allow to return to room temperature before use. Do not expose any of the solutions to temperatures above 37°C. If the kit is used infrequently, storage of the resuspension solution at 4°C is recommended to preserve the RNase.

Section 4

Necessary Supplies

Equipment and supplies to be provided by the customer:

- 1.5–2.0 ml capped microcentrifuge tubes 200
- 95–100% ethanol, or reagent-grade (denatured) ethanol, 100 ml
- Microcentrifuge (12,000 x g)

Additional equipment required for vacuum format:

- Bio-Rad Aurum vacuum manifold with vacuum regulator and column adaptor plate (Cat. #732-6470), or other vacuum manifold with luer fittings (Figure 1).

Note: Please read Section 7, **Instrument Setup and Use for the Column Adaptor Plate** in the Aurum vacuum manifold instruction manual for proper vacuum setup conditions.

- Vacuum source (capability of -20 to -23" Hg required)

Section 5

Guidelines for Using the Aurum Plasmid Mini Kit

Please read the following guidelines before starting the plasmid purification.

Bacterial Growth Guidelines:

- The Aurum plasmid mini kit can process cultures grown in a variety of different broths, such as LB (Luria-Bertani broth), LBG (LB + 2% glycerol), SB (Super Broth) and 2x YT. For optimum performance, LB or LBG is recommended for most strains of *E. coli*. TB (Terrific Broth) cultures generally produce lower plasmid yields of more variable quality and are therefore not recommended.
- For optimum plasmid quality, plasmid propagation in an *endA*⁻ host, such as DH5 α , JM109, or XL1-Blue, is recommended.
- Spectrophotometric determination of culture density is a REQUIREMENT for optimum performance. Optimized concentrations of purified plasmid will generally result with most high copy number constructs, bacterial hosts and

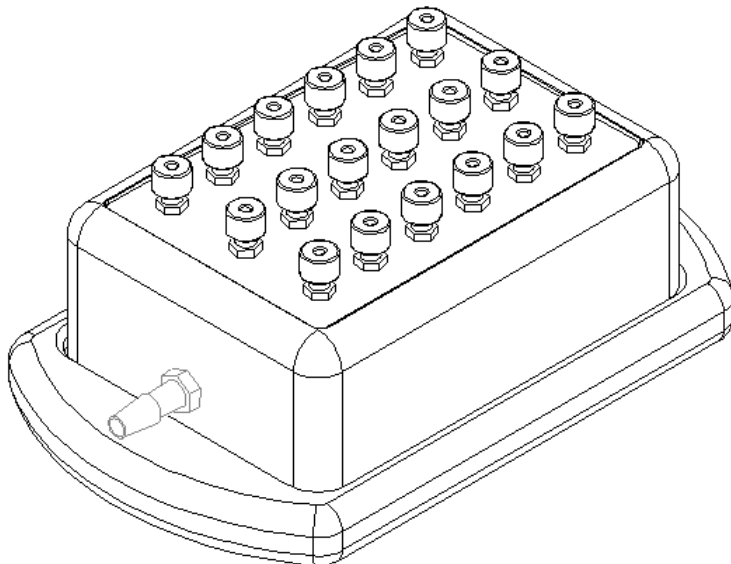


Fig. 1. Aurum vacuum manifold

media when 10–12 OD•ml of bacteria are processed per column, although smaller amounts of culture may also be processed.

To determine the density of a bacterial culture (OD₆₀₀), combine 50 µl of bacterial culture with 950 µl growth medium (1:20 dilution). Use the growth medium as a blank and take the spectrophotometric reading at λ = 600 nm. Multiply this figure by 20 to calculate the bacterial concentration. Depending upon the OD₆₀₀ value, a specific volume of the culture will be selected to provide an optimum amount of bacteria for processing. To calculate the volume of bacterial culture required for plasmid purification, use the following equation:

$$(\text{OD}_{600} \text{ of undiluted culture}) * x (\text{culture volume in ml}) = \# \text{OD} \bullet \text{ml}$$

For example, 12 OD•ml of bacteria would require 2 ml of an undiluted culture with an OD₆₀₀ = 6.

- The protocol is designed to process up to 12 OD•ml of bacterial host.

*OD₆₀₀ is equivalent to approximately 8 x 10⁸ cells/ml

Vacuum Guidelines:

- The recommended operating range is -20 to -23 inches of mercury (" Hg). Do not exceed -25" Hg when performing this protocol. A vacuum regulator is required to establish the appropriate vacuum pressure (Figure 2).

Table 1. Pressure unit conversions

| To convert from inches of mercury (" Hg) to: | Multiply by: |
|---|---------------------|
| millimeters of mercury or torr (mm Hg, torr) | 25.4 |
| millibar (mbar) | 33.85 |
| atmospheres (atm) | 0.03342 |
| pounds per square inch (psi) | 0.4912 |
| kilopascals (kPa) | 3.385 |

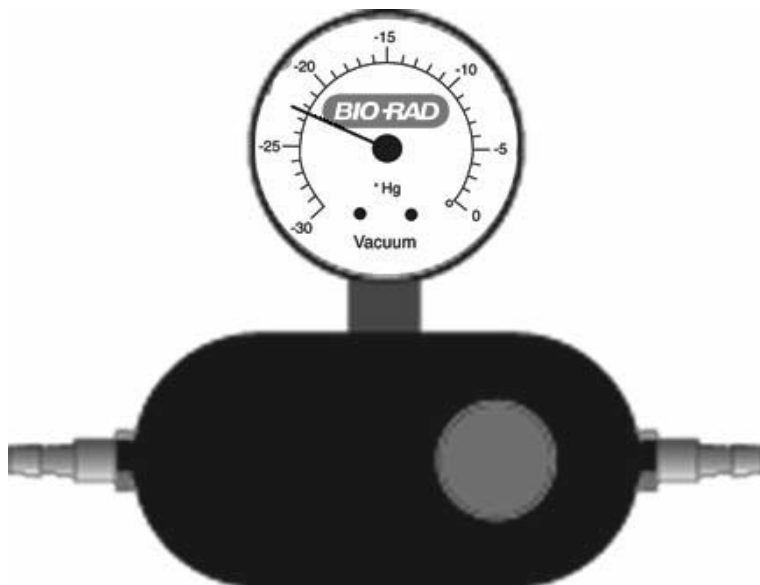


Fig. 2. Vacuum regulator with clear representation of the gauge

Section 6 Protocol

Vacuum Format

This procedure requires the Bio-Rad Aurum vacuum manifold and column adaptor plate (Cat. #732-6470), or any vacuum manifold with luer fittings. For proper vacuum setup conditions, please read **Instrument Setup and Use for the Column Adaptor Plate** in the vacuum manifold instruction manual, and see Fig. 4. All centrifugation steps are performed at maximum speed (12,000 x g) under ambient conditions, with any commercially available microcentrifuge which can accommodate 1.5 ml and 2.0 ml tubes. Please read the section "Guidelines for Using the Aurum Plasmid Mini Kit" before proceeding.

1. Transfer up to 12 OD•ml of plasmid-containing bacterial host to a 1.5–2.0 ml capped microcentrifuge tube (not provided). Pellet the cells by centrifugation for 1 min. Remove all supernatant by decanting or pipetting.
2. Add 250 μ l of resuspension solution and vortex or pipet up and down until the cell pellet is completely resuspended.
3. Add 250 μ l of lysis solution and mix by inverting the capped tube briskly 6–8 times. DO NOT VORTEX OR SHAKE. The solution should become viscous and slightly clear.

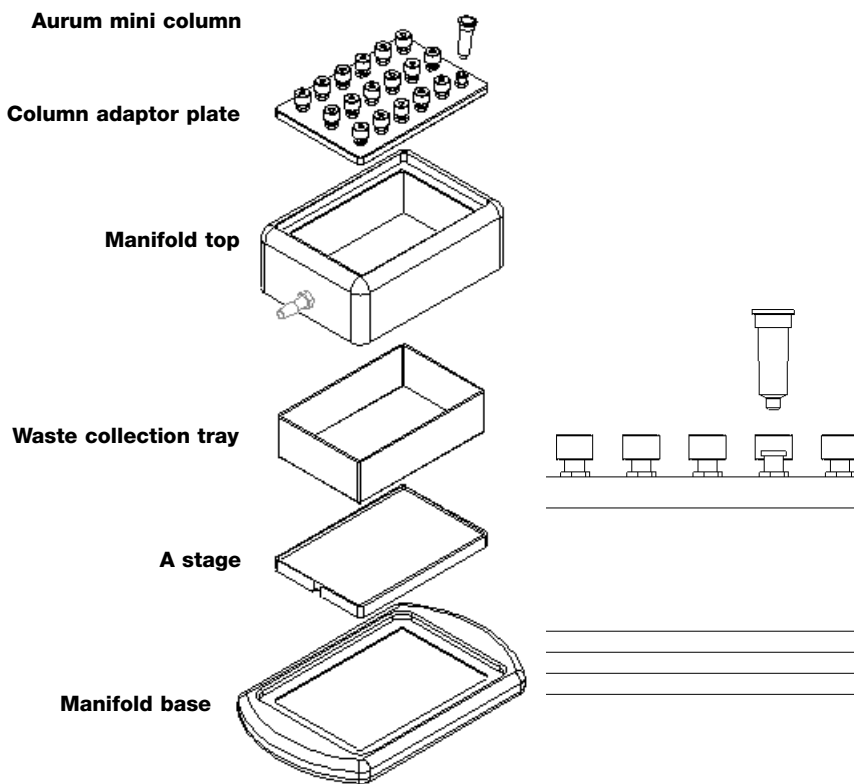


Fig. 3a. Vacuum setup conditions for the Aurum plasmid mini kit

Fig. 3b. Connection of Aurum mini column to column adaptor plate

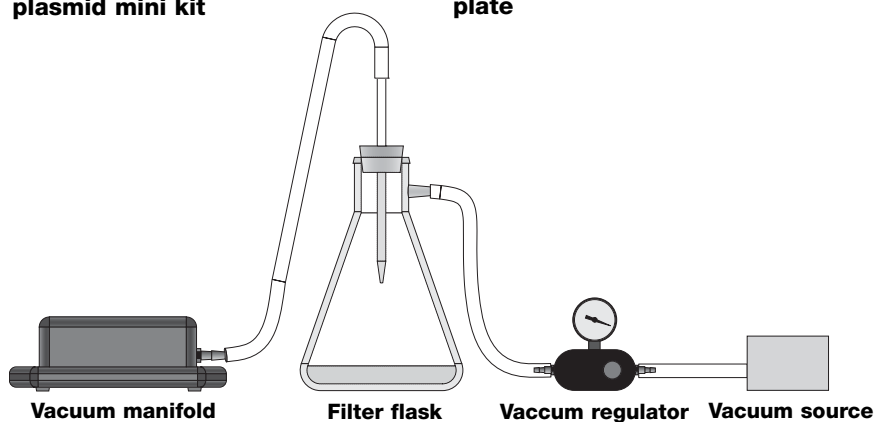


Fig. 4. Vacuum setup conditions

Note: The neutralization solution should be added within 5 min after lysis.

4. Add 350 μ l of neutralization solution and mix by inverting the capped tube briskly 6–8 times. DO NOT VORTEX OR SHAKE. A visible precipitate should form.
5. Centrifuge the neutralized lysate for 5 min. A compact white debris pellet will form along the side or at the bottom of the tube.
6. While centrifuging the lysate, attach a plasmid mini column to the luer fitting of the column adaptor plate on the Aurum vacuum manifold or to a compatible vacuum manifold. The vacuum source should be turned off, and the vacuum regulator should be completely open.
7. By decanting or pipetting, transfer the cleared lysate from step 5 to the plasmid mini column. Turn the vacuum on and adjust to -20 to -23" Hg by closing the vacuum regulator. Continue to apply vacuum until all of the lysate has passed through the column. Open the vacuum regulator until the gauge indicates 0" Hg.
8. The wash solution is supplied as a 5x concentrate. Add 4 volumes (100 ml) of 95–100% ethanol or reagent-grade (denatured) ethanol before initial use.
9. Add 750 μ l of wash solution to the column and close the vacuum regulator dial until the gauge indicates -20 to -23" Hg. Continue to apply the vacuum until all wash solution has passed through the columns. Open the vacuum regulator until the gauge indicates 0" Hg.
10. Transfer the plasmid mini column to a 2 ml capless wash tube (provided) and centrifuge for 1 min to remove residual wash solution.
11. Transfer the plasmid mini column to a 1.5–2.0 ml capped microcentrifuge tube (not provided). Add 50 μ l of elution solution onto the membrane stack at the base of the column and allow 1 min for the solution to saturate the membranes. Centrifuge for 1 min to elute the plasmid.
12. Discard the mini column and store the eluted DNA at 4°C.

A protocol overview is available (see Figure 5).

Aurum™ Plasmid Mini Kit: Vacuum Format

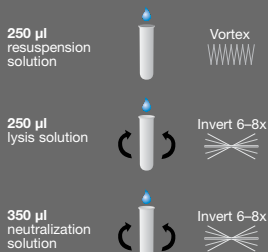


Protocol Overview

For complete protocol, consult instruction manual.

Growth and Isolation

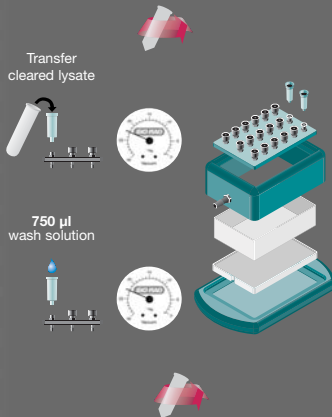
1. Grow 1–5 ml bacterial culture overnight or 16 hr.
2. Measure A_{600} (if higher yield required).
3. Transfer an appropriate volume of culture to a capped 2 ml tube. Centrifuge and decant supernatant.
4. Add 250 μ l resuspension solution; vortex.
5. Add 250 μ l lysis solution; invert 6–8x.
6. Add 350 μ l neutralization solution; invert 6–8x.
7. Centrifuge 5 min to pellet cell debris.



Purification on Aurum or Comparable Manifold

(See exploded view for proper setup of manifold.)

8. Transfer cleared lysate (supernatant) to mini spin/vac column.
9. Apply vacuum at -20 to -23 " Hg to bind plasmid DNA. Turn vacuum off.
10. Add 750 μ l wash solution and reapply vacuum until all liquid has passed through column.
11. Transfer mini spin/vac column to a 2 ml wash tube. Spin **1 min** to remove residual wash.



Collection of Purified Samples

12. Transfer mini spin/vac column to a clean 1.5–2.0 ml capped tube.
13. Add 50 μ l elution solution. Let stand 1 min and then centrifuge 1 min to elute.
14. Purified DNA is ready to use or can be stored at 4°C.



Aurum Plasmid Mini Kit: Cat. #732-6400

For more information, call Technical Services at 1-800-424-6723.

Bulletin 2663 US/EG Rev B 01-568 0901
4110116 Rev A

Fig. 5. Aurum plasmid mini protocol overview: vacuum format

Spin Format

The Aurum plasmid mini kit can be used with any commercially available microcentrifuge that can accommodate 1.5 ml and 2.0 ml tubes. All centrifugation steps are performed at maximum speed (12,000 x g) under ambient conditions. Please read the previous section "Guidelines for Using the Aurum Plasmid Mini Kit" before proceeding.

1. Transfer up to 12 OD•ml of plasmid-containing bacterial host to a 1.5–2.0 ml capped microcentrifuge tube (not provided). Pellet the cells by centrifugation for 1 min. Remove all supernatant by decanting or pipetting.
2. Add 250 μ l of resuspension solution and vortex or pipet up and down until the cell pellet is completely resuspended.
3. Add 250 μ l of lysis solution and mix by inverting the capped tube briskly 6–8 times. DO NOT VORTEX OR SHAKE. The solution should become viscous and slightly clear.

Note: The neutralization solution should be added within 5 min. after lysis.

4. Add 350 μ l of neutralization solution and mix by inverting the capped tube briskly 6–8 times. DO NOT VORTEX OR SHAKE. A visible precipitate should form.
5. Centrifuge the neutralized lysate for 5 min. A compact white debris pellet will form along the side or at the bottom of the tube. The supernatant or cleared lysate contains the plasmid DNA.
6. While centrifuging the lysate, insert a plasmid mini column into a 2 ml capless wash tube (provided).
7. By decanting or pipetting, transfer the cleared lysate from step 5 to the plasmid mini column. Centrifuge for 1 min.
8. The wash solution is supplied as a 5x concentrate. Add 4 volumes (100 μ l) of 95–100% ethanol or reagent-grade (denatured) ethanol before initial use.
9. Remove the plasmid mini column from the wash tube. Discard the filtrate from the tube, and replace the column into the same wash tube. Add 750 μ l of wash solution and centrifuge for 1 min.
10. Discard the wash solution from the tube, and replace the column into the same wash tube. Centrifuge for 1 additional minute to remove residual wash solution.
11. Transfer the plasmid mini column to a 1.5–2.0 ml capped microcentrifuge tube (not provided). Add 50 μ l of elution solution onto the membrane stack at the base of the column and allow 1 min for the solution to saturate the membranes. Centrifuge for 1 min to elute the plasmid.
12. Discard the mini column and store the eluted DNA at 4°C.

A protocol overview is available (see Figure 6).

Aurum™ Plasmid Mini Kit: Spin Format

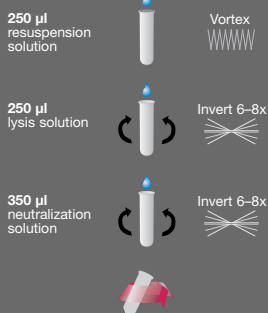


Protocol Overview

For complete protocol, consult instruction manual.

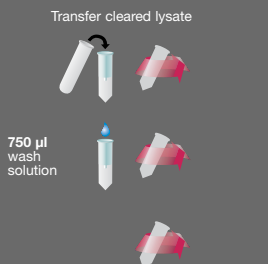
Growth and Isolation

1. Grow 1–5 ml bacterial culture overnight or 16 hr.
2. Measure A_{600} (if higher yield required).
3. Transfer an appropriate volume of culture to a capped 2 ml tube. Centrifuge and decant supernatant.
4. Add 250 μ l resuspension solution; vortex.
5. Add 250 μ l lysis solution; invert 6–8x.
6. Add 350 μ l neutralization solution; invert 6–8x.
7. Centrifuge 5 min to pellet cell debris.



Purification

8. Transfer cleared lysate (supernatant) to mini spin/vac column.
9. Centrifuge 1 min to bind plasmid DNA. Decant flow-through.
10. Add 750 μ l wash solution and centrifuge 1 min. Decant flow-through.
11. Centrifuge additional 1 min to remove residual wash solution.



Collection of Purified Samples

12. Transfer mini spin/vac column to a clean 1.5–2.0 ml capped tube.
13. Add 50 μ l elution solution. Let stand 1 min and then centrifuge 1 min to elute.
14. Purified DNA is ready to use or can be stored at 4°C.



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at 1-800-424-6723.

Fig. 6. Aurum plasmid mini protocol overview: spin format

Section 7

Troubleshooting Guide

| Problem | Possible Cause | Possible Solution |
|---------------------------|---|--|
| Low plasmid yields | Low copy number plasmid | Use high copy number constructs |
| | Poor plasmid propagation in culture | Inoculate large-scale cultures with overnight cultures generated from fresh colonies grown on a selective medium Determine optimum growth and plasmid propagation times for culture depending upon host, broth, etc. Check age and concentration of antibiotic |
| | Excessive amount of bacteria processed | Determine OD ₆₀₀ of culture and reduce OD•ml of bacteria processed to ensure efficient lysis |
| | Incomplete resuspension of bacteria pellet | Ensure that the bacterial pellet is completely resuspended prior to addition of lysis solution |
| | Excessive drying of column membranes prior to elution | See protocol. Do not exceed 1 min centrifugation. |
| | Insufficient ethanol added to wash solution concentrate | Check that correct amount was added |

| Problem | Possible Cause | Possible Solution |
|------------------------------------|--|--|
| Bacterial DNA contamination | Excessive amount of bacteria processed | Determine OD ₆₀₀ of culture and do not exceed 12 OD•ml of bacteria processed |
| | Excessive agitation of lysate | Do not shake or vortex lysate after addition of lysis solution |
| RNA contamination | Excessive amount of bacteria processed | Determine OD ₆₀₀ of culture and do not exceed 12 OD•ml of bacteria processed |
| | Compromised RNase activity due to age or storage conditions | Replace kit |
| Low A_{260/280} | Excessive amount of bacteria processed | Determine OD ₆₀₀ of culture and do not exceed 12 OD•ml of bacteria processed |
| | Incomplete suspension of bacterial pellet | Ensure that the bacterial pellet is completely resuspended prior to addition of lysis solution |
| | Incomplete mixing of resuspension, lysis, and neutralization solutions | Invert briskly 6–8 times after the addition of each reagent; check to ensure homogeneous mixing |
| | Incomplete washing of column membranes | Use recommended wash solution volumes. Add correct amount of ethanol to wash solution concentrate. |

| Problem | Possible Cause | Possible Solution |
|--|---|---|
| More than one band on analytical gel | Presence of multimers – typical and variable depending upon plasmid, bacterial host, etc. | Try different host or growth conditions |
| | Nicking due to endonuclease activity | Use <i>endA</i> ⁻ host such as JM109, DH5 α , or XL1-Blue |
| | Prolonged exposure to alkaline conditions | Add neutralization solution within 5 min after addition of lysis solution |
| Plasmid nicked/ degraded | Use of <i>endA</i> ⁺ bacterial host | Use <i>endA</i> ⁻ host such as JM109, DH5 α , or XL1-Blue |
| Plasmid prep performs poorly in enzymatic reactions | Plasmid nicked, degraded or denatured | Check integrity of plasmid on an analytical gel |
| | | Use <i>endA</i> ⁻ host |

Section 8

Ordering Information

| Catalog # | Description |
|-----------|---|
| 732-6470 | Aurum Vacuum Manifold , includes column adaptor plate, 4 replacement luer caps, A and B stages, waste collection tray, vacuum regulator and gauge, tubing, protocol overview, and instruction manual |
| 732-6400 | Aurum Plasmid Mini Kit , 100 preps, includes 100 plasmid binding columns, 100 capless collection tubes (2.0 ml), reagents, protocol overview, and instruction manual |