Bio-Rad Laboratories, Inc.
2000 Alfred Nobel Drive
Hercules, CA 94547 USA
(510) 741-1000

1-800-424-6723

4110114 Rev A

Aurum™ Plasmid 96 Kit

Instruction Manual

For technical service, call your local Bio-Rad office, or
in the US, call 1-800-4BIORAD (1-800-424-6723)

BIO-RAD

Table of Contents

Section 1
Introduction

The Aurum plasmid 96 kit purifies up to 96 plasmid DNA samples in parallel rapidly and with minimal handling. Based on the alkaline lysis method of purifying plasmid DNA samples, the system uses plasmid-binding membranes and filtration media instead of time-intensive steps normally associated with plasmid purification, such as centrifugations and alcohol precipitations to remove cellular debris and recover plasmid. A unique binary plate configuration is incorporated into the Aurum plasmid 96 kit, minimizing handling as well as the opportunity for cross-contamination and sample loss. Each well will yield up to 20 µg plasmid DNA, and each preparation will assimilate up to 12 OD_{600} of bacterial culture from a variety of different culture media, including LB (Luria-Bertani), LBG (LB+2% glycerol), 2xYT, SB (Super Broth), and TB (Terrific Broth). The Aurum plasmid 96 kit is designed specifically for use with Bio-Rad's Aurum vacuum manifold.

The unique, patent-pending binary plate configuration is comprised of an upper lysate filtration plate joined to a lower plasmid binding plate via a gasket. Unlike other two-plate plasmid purification systems in which the plasmid binding plate is placed inside the manifold during lysate filtration, the binary plates are both positioned on top of the vacuum manifold. As a consequence, the two plates are in intimate contact with each other, eliminating any opportunity for sample loss or cross-contamination during the transfer of plasmid-containing filtrate between the two plates. Moreover, the binary plate configuration streamlines the purification process by obviating the need to insert the DNA binding plate inside the manifold (and then subsequently disassembling the manifold and relocating the plasmid plate), thereby minimizing handling. Following lysate filtration, the lysate filtration plate is simply detached and discarded, leaving the plasmid binding plate on top of the manifold for the subsequent wash step.

Section 2
Kit Components

The Aurum plasmid 96 kit contains the following components:

Binary-plate unit	2
consisting of:	
- Lysate filtration plate (upper)	1
- Plasmid binding plate (lower)	1
Grow block	2
Microtiter collection plate	2
Sealing tape	8 (2 pks. of 4 pieces)
Aurum growth membrane	2

resuspension solution	45 ml
lysis solution	50 ml
neutralization solution	100 ml
wash solution	50 ml (5x concentrate)
elution solution	16 ml

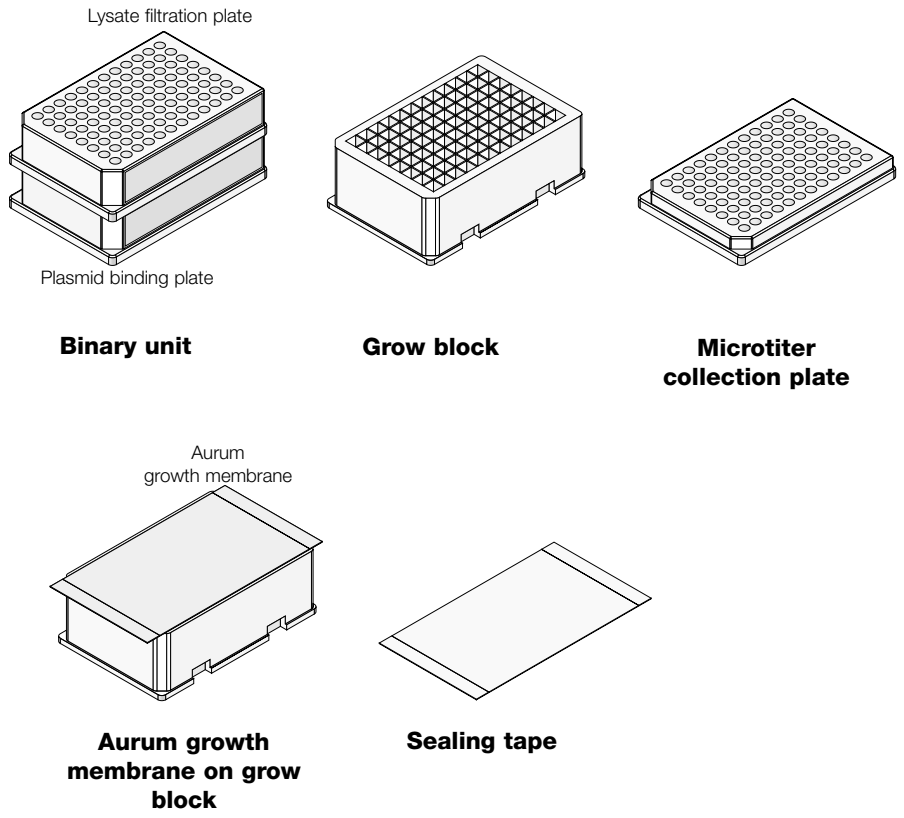

Fig. 1. Aurum plasmid 96 kit components

Section 3
Storage Conditions

Solutions and plates 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.

Section 4
Necessary Supplies

Equipment and supplies to be provided by the customer:

- Aurum vacuum manifold with regulator (Catalog #732-6470)
- Vacuum source (capability of -23" Hg or approximately -800 mbar required)
- Alternate bacterial growth vessels such as microcentrifuge or centrifuge tubes if a grow block is not desired
- Centrifuge with swinging bucket rotor for grow blocks or 96-well microplates, or appropriate rotor if an alternate bacterial growth vessel is used
- Multichannel pipet (recommended)
- Reagent reservoirs (recommended)

Section 5
Guidelines for Using the Aurum Plasmid 96 Kit

Please read the following guidelines before proceeding with the plasmid purification.

Bacteria Guidelines:

- Each well of the Aurum plasmid 96 kit can process up to 12 OD_{600} of bacterial culture. Optimized concentrations of purified plasmid will generally occur with most high-copy number constructs, bacterial hosts and media types when 6–10 OD_{600} of bacterial culture are processed per well, although smaller amounts of culture may also be processed (Fig. 2).

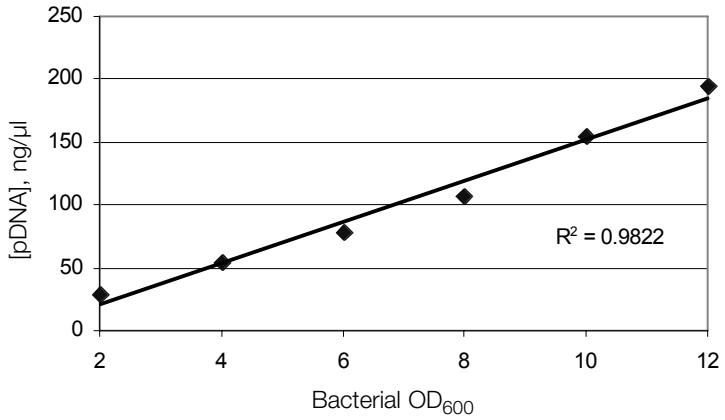

Fig. 2. OD vs. plasmid concentrations

Figure 2. Relationship between OD_{600} of bacteria processed per well and concentration of plasmid DNA obtained using the Aurum plasmid 96 kit. A linear trend is observed between bacterial amount used per well and plasmid DNA sample concentration. 2–12 OD_{600} pGEM/DH5α in LBG were processed in each well. Plasmid DNA samples were eluted with 80 µl elution solution.

To measure the OD_{600} of a culture following bacterial growth, add 50 µl bacterial culture to 950 µl of the appropriate growth medium (1:20 dilution). Blank against the growth medium and take the spectrophotometric reading at λ = 600 nm. Multiply this figure by 20 to calculate the number of OD_{600} per milliliter of culture.

- For optimum plasmid quality, plasmid propagation in an *endA*$^-$ host, such as JM109, DH5α or XL1-Blue, is recommended.
- The Aurum plasmid 96 kit can process bacteria grown in a variety of different broths, such as LB (Luria-Bertani), LBG (LB+2% glycerol), SB (Super Broth) and 2xYT.
- Alternate culture vessels such as microcentrifuge or sterile 15 ml centrifuge tubes may be used instead of the supplied grow blocks for growing the bacteria. When resuspending the bacteria, the appropriate amount of resuspension solution should be added, such that 200 µl of resuspended bacteria will be transferred to each well of a grow block. For example if the bacteria in a microcentrifuge tube is to be aliquotted into three wells, 3 x 200 = 600 µl resuspension solution should be added to the pellet. Do not exceed 12 OD_{600} of bacterial culture per 200 µl of resuspension solution.
- After centrifugation of the bacteria, the intact pellets may be stored at -80°C until needed.

Vacuum Guidelines:

- The recommended operating range is -20–23 inches of mercury (" Hg). A vacuum regulator is **strongly** recommended to establish the appropriate negative pressure.
- The gasket used on the manifold routinely allows the plates to self-seat whenever vacuum is applied, without the necessity to press down on the plates. However, under certain conditions, the application of gentle downward force may occasionally be required to allow the plates to form a seal with the manifold.
- When applying a vacuum to the manifold, increase the negative pressure gradually by slowly closing the vacuum regulator over a 5–10 sec period. This will promote uniform movement of solutions through the wells of the binary plates, as well as minimize sample spraying and cross-contamination during elution.
- As the wells of the filtration plate empty, and during purge steps, the negative pressure within the vacuum manifold may drop to below -15" Hg. This is normal and does not require corrective action.

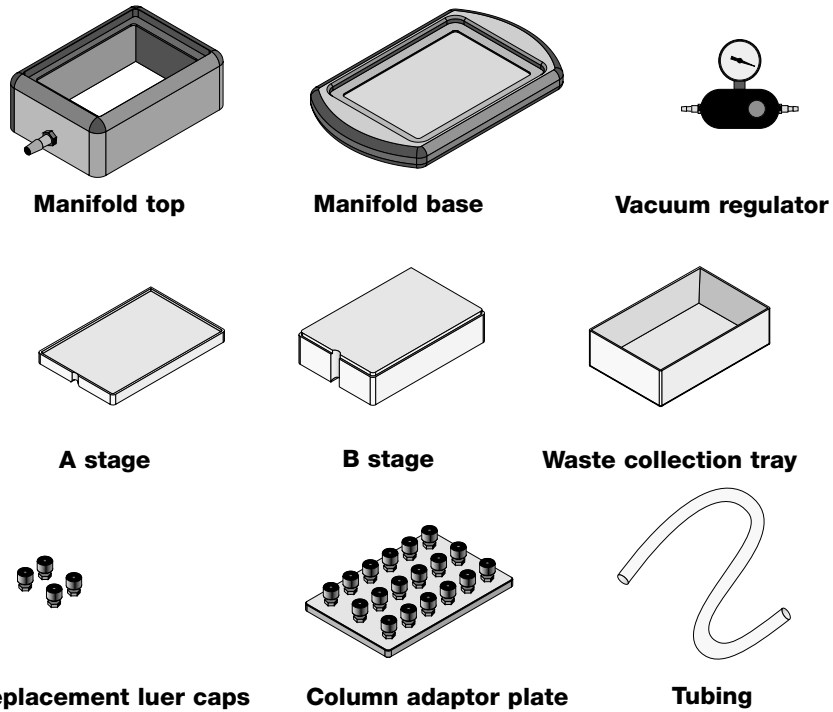

Fig. 3. Aurum vacuum manifold components

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

Elution Guidelines:

- For more concentrated plasmid DNA samples, the volume of elution solution may be reduced to 60–70 µl. Do not use less than 60 µl.
- Apply elution solution directly to the membranes at the base of each well of the plasmid binding plate.

Section 6
Protocol

Please read the section "Guidelines for Using the Aurum Plasmid 96 Kit" before proceeding.

1. Grow the bacteria in a 96-well grow block. Add 1.0–1.3 ml growth medium with appropriate antibiotic to each well of the grow block. Inoculate each well with a single bacterial colony picked from a freshly-streaked agar plate. Cover the grow block with an Aurum growth membrane and incubate overnight at 37°C and shaking at 250–300 rpm.
2. Centrifuge the grow block at 1,500 x *g* for 10 min to pellet the bacteria. Invert the grow block, decant the supernatant, and blot the inverted grow block with paper towels to remove residual liquid.
3. Add 200 µl of resuspension solution to each grow block well. Seal the block with sealing tape, smoothing out any wrinkles, and resuspend the bacterial pellet completely by vortexing or by other agitation.
4. Remove the sealing tape. Add 200 µl of lysis solution into each well. Seal the grow block with sealing tape and invert the block 6–8 times to lyse the bacteria. DO NOT VORTEX OR SHAKE. The lysate should become viscous.

   Note: The neutralization solution (step 5) should be added to the bacterial lysate within 5 min after adding the lysis solution.
5. Remove the sealing tape. Add 300 µl of neutralization solution into each well. Seal the block with fresh sealing tape and invert 6–8 times to mix. DO NOT VORTEX OR SHAKE. The bacterial chromosomal DNA and cellular debris will form a visible white precipitate.
6. Set up the Aurum vacuum manifold and one pre-assembled binary-plate unit in the following manner:
   a. Remove the manifold top from the manifold base and position the A stage into the recess in the manifold base, with the side marked "A" facing up.
   b. Place the waste collection tray into the shallow depression in the top of the A stage.
   c. Replace the manifold top onto the manifold base. Check that the manifold top establishes a uniform seal with the manifold base.
   d. Place a binary-plate unit into the rectangular recess in the manifold top, with the yellow lysate filtration plate on top.

7. With the vacuum turned off, transfer the lysate from each well of the grow block into a corresponding well of the lysate filtration plate. The vacuum regulator dial should be completely open (turned fully counterclockwise).
8. Turn the vacuum on and gradually increase the negative pressure to -20–23" Hg over a 5–10 sec period by slowly turning the vacuum regulator dial clockwise. After all wells have emptied, continue the vacuum for an additional 5 min. Open the vacuum regulator fully. Check that the regulator gauge reads approximately 0" Hg.

   Note: Gradual application of negative pressure is required to ensure uniform flow of lysates through all 96 wells of the lysates filtration plate.
9. Detach and discard the upper lysate filtration plate. Do not remove the plasmid binding plate from the vacuum manifold.
10. The wash solution is provided as a 5x concentrate. Add 4 volumes (200 ml) 95–100% ethanol to the wash solution concentrate before initial use.
11. Add 750 µl of wash solution into each well of the plasmid binding plate. Gradually increase the negative pressure to -20–23" Hg over a 5–10 sec period by slowly closing the vacuum regulator. After all wells have emptied, continue the vacuum for an additional 5 min to purge the wells of residual wash solution. When completed, open the vacuum regulator until the gauge reads approximately 0" Hg.

    Note: Do not allow the vacuum purge to continue for longer than 5 min as excessive drying of the plate membranes may occur.
12. Set up the Aurum vacuum manifold for elution in the following manner:
    a. Remove the plasmid binding plate from the manifold and tap or press against several layers of paper towels to remove any attached droplets of wash solution. Remove the top of the manifold and remove and empty the waste collection tray and A stage.
    b. Place the B stage on the manifold base, with the side marked "B" facing up.
    c. Place a sample collection plate on top of the B stage. Check that the sample collection plate base sits firmly in the groove around the B stage edge.
    d. Replace the manifold top and ensure that it is uniformly seated against the manifold base. Place the plasmid binding plate into the gasketed recess in the manifold top.

13. Pipette 80 µl of elution solution on to the membranes at the base of each well of the plasmid binding plate and allow 1 min for the solution to saturate the membranes. Gradually increase the negative pressure to -20–23" Hg over a 5–10 second period by slowly closing the vacuum regulator. Continue the vacuum for 5 min. Turn off the vacuum source and open the vacuum regulator.

    Note: Gradual application of negative pressure is required to prevent sample spraying and cross-contamination. For more concentrated plasmid DNA samples, the volume of elution solution may be reduced to 60–70 µl. However, do not elute with less than 60 µl.

    The eluted plasmid samples in the microtiter collection plate can be used immediately in cycle sequencing reactions or any other application. Alternatively, the microtiter collection plate can be sealed with sealing tape and stored for later use.

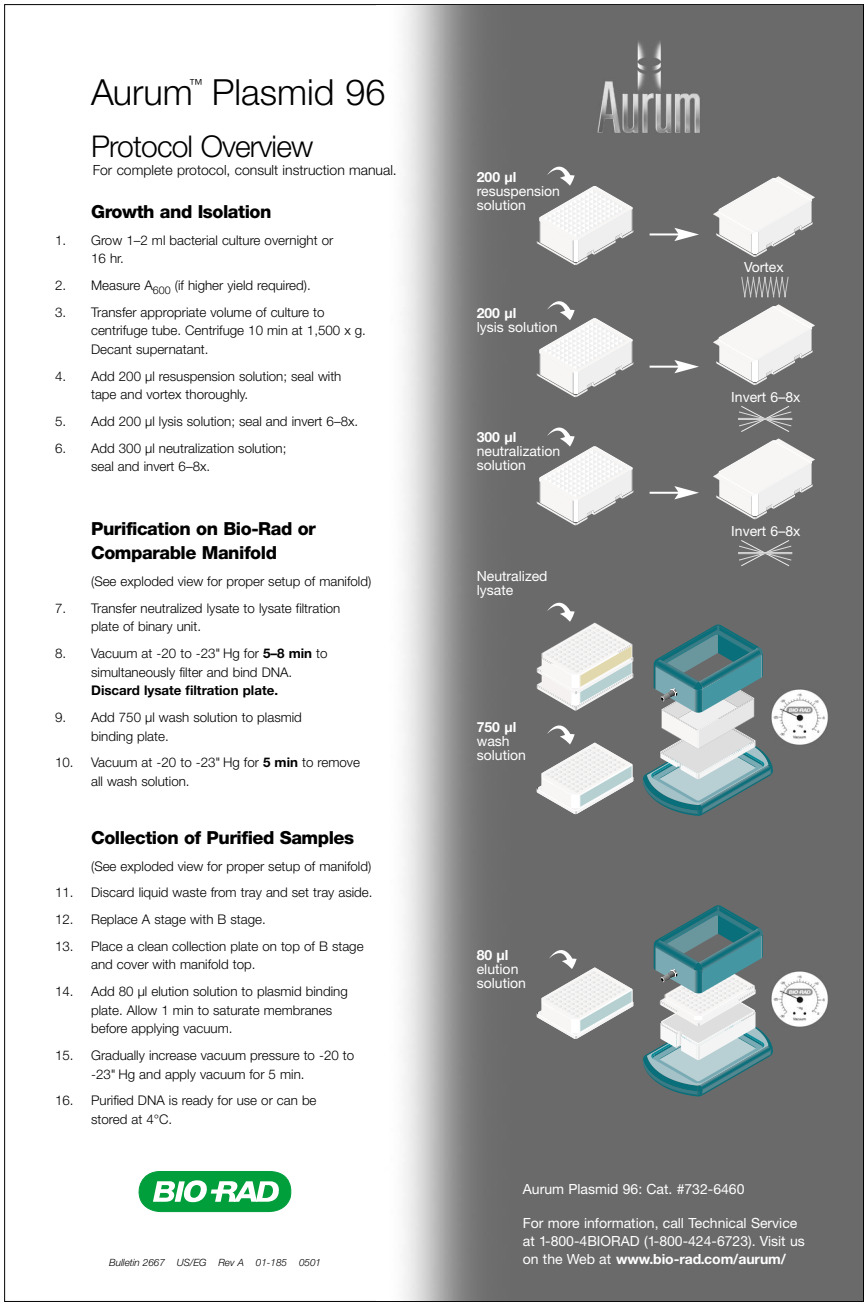

Fig. 4. Aurum plasmid 96 protocol overview

Section 7
Troubleshooting Guide

Problem	Possible Cause	Possible Solution
Difficulty achieving -20–23" Hg negative pressure	Purge step in protocol	If closure of wells with sealing tape restores negative pressure, no corrective action required. However do not leave closed.
	Open vacuum regulator	Turn control dial fully clockwise to close regulator
	Plates not sealing	Press down gently on binary unit or plasmid binding plate to seat
	Manifold top not seated properly on base	Ensure that the manifold top is seated properly on base
	Residue on gasket	Rinse manifold and gasket with distilled water
	Gasket detached	Press down to reestablish uniform adhesive contact or replace
	Worn gasket	Replace gasket
Lysate filtration exceeds 5 min	Insufficient negative pressure	See Problem "Difficulty achieving -20–23" Hg negative pressure"
	Excessive amount of bacteria processed	Determine OD_{600} of culture and do not exceed recommended amounts

Problem	Possible Cause	Possible Solution
	Excessive bacterial debris due to use of growth medium or conditions	Switch to alternate bacterial medium
	Incomplete mixing of sample during resuspension, lysis, or neutralization	Invert briskly to mix, but do not vortex or shake
Cross-contamination among adjacent wells	Abrupt application of negative pressure during elution	Increase negative pressure gradually (5–10 sec) using the vacuum regulator
	Residual wash buffer on drip directors	Blot plasmid binding plate drip directors with paper towels
Low or highly variable eluate volumes among wells	Elution solution applied to plasmid binding plate well walls	Apply elution solution directly to membranes at base of each well
	Plasmid binding plate not seated properly	Set plate properly, and press down gently to seat
	Eluate is splattering	Increase negative pressure gradually (5–10 sec) using a vacuum regulator
Low plasmid yield	Low copy number plasmid	Use medium or high copy number plasmid
	Growth conditions not optimized	Optimize growth conditions (broth type, incubation time, etc.)
	Poor plasmid propagation	Inoculate fresh overnight cultures with single colonies from a freshly streaked plate

Problem	Possible Cause	Possible Solution
Genomic DNA contamination	Excessive force applied during or after bacterial lysis	Invert lysate gently; DO NOT VORTEX OR SHAKE
	Excessive amount of bacteria processed	Determine OD_{600} of culture and do not exceed recommended amounts
RNA contamination	Excessive amount of bacteria processed	Determine OD_{600} of culture and do not exceed recommended amounts
	Resuspension solution improperly stored	Store resuspension solution at 4°C
Low $A_{260/280}$ ratio (<1.7)	Excessive amount of bacteria processed	Determine OD_{600} of culture and do not exceed recommended amounts
	Incorrect volumes of solutions used	Use correct volumes of resuspension, lysis, and neutralization solutions
	Incomplete resuspension of bacterial pellet	Resuspend bacterial pellet thoroughly by vortexing or pipetting up and down
	Incomplete mixing of lysate during lysis or neutralization	Invert briskly to mix, but do not vortex or shake
	Incomplete washing of membranes prior to elution	Perform additional washes

Problem	Possible Cause	Possible Solution
Multiple bands observed on analytical gel	Nicked plasmid	Corrective action may not be necessary—plasmid will perform well in many applications
	Genomic DNA contamination	See Problem "Genomic DNA contamination"
	Prolonged exposure of plasmid to alkaline conditions	Add neutralization solution within 5 minutes of lysis
Plasmid is degraded	*endA*$^+$ strain used	Use *endA*$^-$ strain (e.g., JM109, DH5α, XL1-Blue)
Plasmid prep performs poorly in enzymatic reactions	Proteinaceous debris in prep (if $A_{260/280}$ <1.7)	See Problem "Low $A_{260/280}$ ratio"
	Plasmid is degraded	Use *endA*$^-$ strain (e.g. JM109, DH5α, XL1-Blue)
	Ethanol contamination in prep (eluate volumes >55 µl)	Add 1–3 min to the purge time after the wash step
		Remove all traces of wash solution from the drip directors of the plasmid binding plate by blotting on paper towels prior to elution

Section 8
Ordering Information

Catalog #	Description
732-6460	Aurum plasmid 96 kit, 2 x 96 well preps, includes 2 grow blocks, 2 grow membranes, 2 binary units (2 lysate filtration plates and 2 plasmid binding plates), 2 microtiter collection plates, reagents, protocol overview and instruction manual.
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), 100 capped sample tubes (1.5 ml), reagents, protocol overview and instruction manual.
732-6440	Aurum plasmid midi kit, 20 preps, includes 20 lysate filtration columns, 20 plasmid binding columns, 20 adaptor caps, reagents, protocol overview and instruction manual.
732-6450	Aurum plasmid maxi kit, 10 preps, includes 10 lysate filtration columns, 10 plasmid binding columns, 10 adaptor caps, reagents, protocol overview and instruction manual.