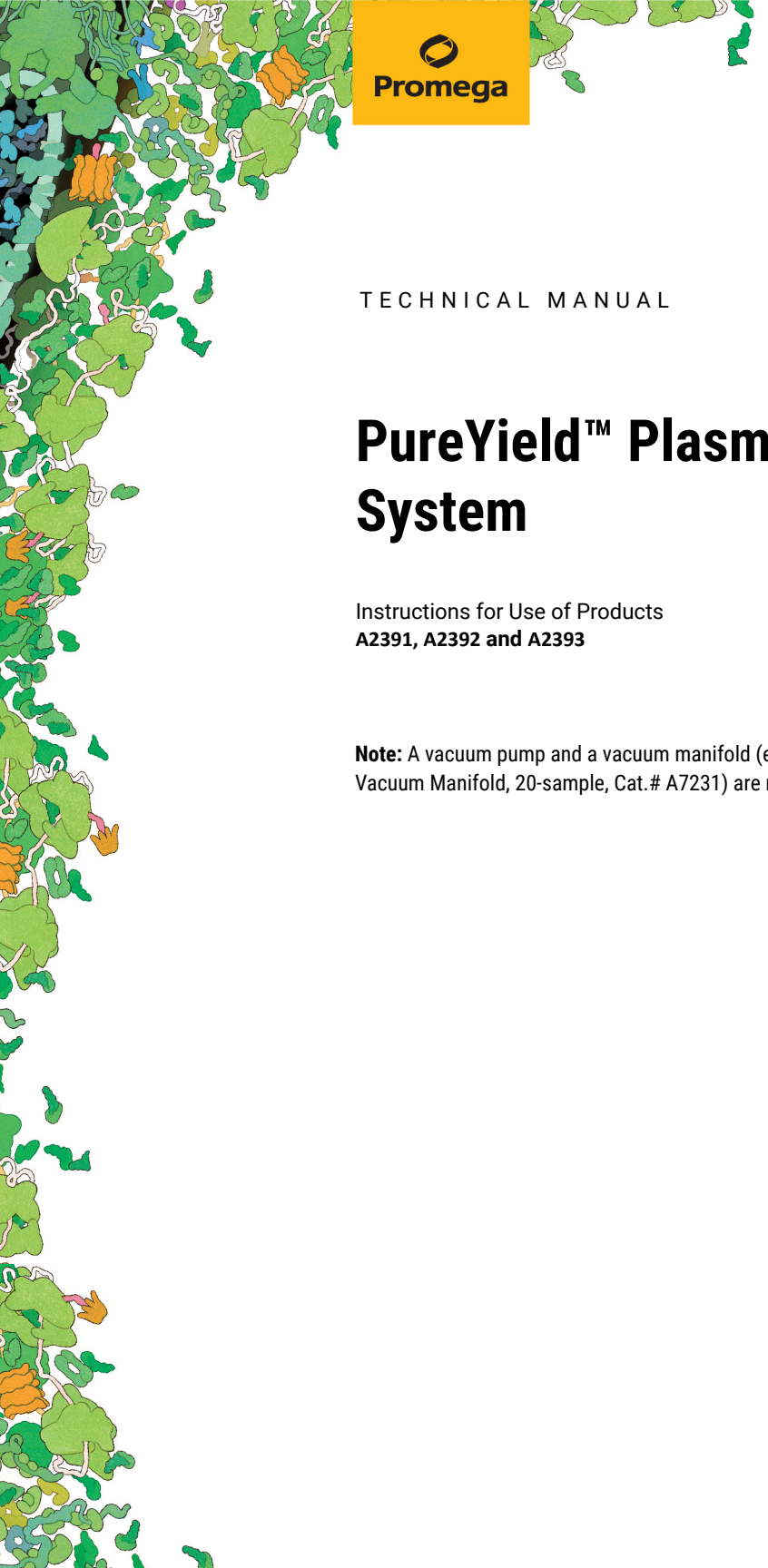

TECHNICAL MANUAL

PureYield™ Plasmid Maxiprep System

Instructions for Use of Products
A2391, A2392 and A2393

Note: A vacuum pump and a vacuum manifold (e.g., the Vac-Man® Laboratory Vacuum Manifold, 20-sample, Cat.# A7231) are required for use with this system.

Revised 9/23
TM280

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PureYield™ Plasmid Maxiprep System

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

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1. Description

As research moves from DNA sequencing to analyzing gene function, the need has increased for rapid methods by which to isolate large quantities of high-quality plasmid DNA. The PureYield™ Plasmid Maxiprep System[a] is designed to isolate high-quality plasmid DNA for use in eukaryotic transfection and in cell-free expression experiments. The system provides a rapid method for purification using a silica-membrane column. Plasmid DNA can be purified in approximately 60 minutes, greatly reducing the time spent on purification compared to silica-resin or other membrane column methods.

The PureYield™ Plasmid Maxiprep System also incorporates a unique Endotoxin Removal Wash designed to remove substantial amounts of protein, RNA and endotoxin contaminants from purified plasmid DNA, and improve the robustness of sensitive applications such as eukaryotic transfection, in vitro transcription and cell-free expression. Purification is achieved without isopropanol precipitation of purified plasmid DNA, providing rapid purification as well as a high concentration of pure plasmid DNA. The PureYield™ Plasmid Maxiprep System purifies up to 1mg of plasmid DNA with an A_{260}/A_{280} >1.7 from 250ml of overnight bacterial culture, transformed with a high-copy-number plasmid.

The PureYield™ System requires a vacuum pump and manifold (e.g., the Vac-Man® Laboratory Vacuum Manifold, 20-sample [Cat.# A7231]), a centrifuge with a fixed-angle rotor for lysate clearing and a tabletop centrifuge with a swinging bucket rotor for elution by centrifugation. Alternatively and for optimal DNA recovery and yield, elution can be performed by vacuum, with the Eluator™ Vacuum Elution Device (Cat.# A1071; see Section 4.B). Use of the Eluator™ Elution Device eliminates the need for a tabletop centrifuge and swinging bucket rotor.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
PureYield™ Plasmid Maxiprep System	**2 preps**	**A2391**

Each system contains sufficient reagents for 2 × 250ml preps. Includes:

- 32ml Cell Resuspension Solution (CRA)
- 32ml Cell Lysis Solution (CLA)
- 30ml Neutralization Solution (NSB)
- 8.1ml Endotoxin Removal Wash
- 18ml Column Wash
- 3.75ml Nuclease-Free Water
- 2 each PureYield™ Clearing Columns
- 2 each PureYield™ Maxi Binding Columns

PRODUCT	SIZE	CAT.#
PureYield™ Plasmid Maxiprep System	**10 preps**	**A2392**

Each system contains sufficient reagents for 10 × 250ml preps. Includes:

- 125ml Cell Resuspension Solution (CRA)
- 125ml Cell Lysis Solution (CLA)
- 130ml Neutralization Solution (NSB)
- 36.5ml Endotoxin Removal Wash
- 82ml Column Wash
- 25ml Nuclease-Free Water
- 10 each PureYield™ Clearing Columns
- 10 each PureYield™ Maxi Binding Columns

PRODUCT	SIZE	CAT.#
PureYield™ Plasmid Maxiprep System	**25 preps**	**A2393**

Each system contains sufficient reagents for 25 × 250ml preps. Includes:

- 315ml Cell Resuspension Solution (CRA)
- 315ml Cell Lysis Solution (CLA)
- 315ml Neutralization Solution (NSB)
- 85.3ml Endotoxin Removal Wash
- 210ml Column Wash
- 50ml Nuclease-Free Water
- 25 each PureYield™ Clearing Columns
- 25 each PureYield™ Maxi Binding Columns

Storage Conditions: Store all system components at room temperature (+15°C to +30°C).

3. Equipment, Supplies and Preparation of Solutions

Materials to Be Supplied by the User

(Solution compositions are provided in Section 5.D.)

- isopropanol
- ethanol, 95%
- tabletop centrifuge
- swinging bucket rotor
- disposable plastic 50ml screw-cap tubes (Corning Cat.# C4558 or BD Falcon™ Cat.# 352070)
- vacuum pump, single- or double-stage, producing approximately 650mm Hg (25.6 inches Hg) of pressure (see table for pressure comparisons)
- fixed-angle centrifuge
- vacuum manifold (e.g., Vac-Man® Laboratory Vacuum Manifold, 20-sample [Cat.# A7231])
- **optional:** Eluator™ Vacuum Elution Device (Cat.# A1071)

Comparison of Inches of Hg to Other Pressure Measurements.

1 Inch Hg	25.6 Inches Hg
3.386kPa	86.7kPa
25.4Torr	650Torr
0.0334atm	0.855atm
0.491psi	12.57psi
2.54cm Hg	65.0cm Hg
33.86mbar	867mbar

Before lysing cells and purifying DNA, Endotoxin Removal Wash and Column Wash must be prepared as described below (cap tightly after additions):

Endotoxin Removal Wash Preparation

- **2 preps system (Cat.# A2391):**
  Add 5.5ml of isopropanol to the Endotoxin Removal Wash before use.
- **10 preps system (Cat.# A2392):**
  Add 24ml of isopropanol to the Endotoxin Removal Wash before use.
- **25 preps system (Cat.# A2393):**
  Add 57ml of isopropanol to the Endotoxin Removal Wash before use.

Column Wash Preparation

- **2 preps system (Cat.# A2391):**
  Add 30ml of 95% ethanol to the Column Wash before use.
- **10 preps system (Cat.# A2392):**
  Add 137ml of 95% ethanol to the Column Wash before use.
- **25 preps system (Cat.# A2393):**
  Add 350ml of 95% ethanol to the Column Wash before use.

4. PureYield™ Plasmid Maxiprep System Protocol

4.A. Preparation and Lysis of Bacterial Cell Cultures

Note: Throughout the remainder of this document the supplied Cell Resuspension Solution (CRA), Cell Lysis Solution (CLA) and Neutralization Solution (NSB) are referred to as Cell Resuspension Solution, Cell Lysis Solution and Neutralization Solution, respectively.

Note: Perform all lysis steps at room temperature.

1. Grow 100–250ml of transformed *E. coli* bacterial cell culture overnight (16–21 hours) at optimal culture conditions.
2. Pellet the cells by centrifugation at 5,000 × *g* for 10 minutes at room temperature, and discard supernatant. Drain tubes on a paper towel to remove excess media.
3. Resuspend the cell pellets in 12ml of Cell Resuspension Solution.

   Note: Make sure that cell resuspension is complete.
4. Add 12ml of Cell Lysis Solution and mix by gently inverting the tube 3–5 times. Incubate for 3 minutes at room temperature.

   Note: If the Cell Lysis Solution becomes too cold, SDS precipitation may occur, which could result in poor cell lysis. If a precipitate has formed, warm the Cell Lysis Solution to 37°C with gentle shaking before use.
5. Add 12ml of Neutralization Solution to the lysed cells, cap the tube and mix by gently inverting the tube 10–15 times. It is important to completely mix the solution to ensure complete precipitation of cellular debris. A well-mixed solution will appear flocculant, with small- to medium-sized clumps. If the solution has not been mixed thoroughly it will instead have a coagulated appearance.
6. Centrifuge the lysate at 14,000 × *g* for 20 minutes at room temperature in a fixed-angle rotor. This centrifugation will pellet the bulk of the cellular debris. If debris remains it can be removed using a PureYield™ Clearing Column.

   Alternatively, the lysate may be centrifuged at 7,000 × *g* for 30 minutes.

   When the spin is complete, examine the cleared lysate. It should be clear, with small amounts of cell debris. If the lysate is cloudy, it should be filtered through Miracloth™ before continuing.

4.B. DNA Purification

Note: Perform all purification and elution steps at room temperature.

1. Assemble a column stack by placing a blue PureYield™ Clearing Column on the top of a white PureYield™ Maxi Binding Column. Place the assembled column stack onto the vacuum manifold (Figure 1).

   Note: Alternatively, if the lysate is completely clear of cloudiness and debris, the blue clearing column may be omitted, and the lysate can be poured directly onto the binding column.

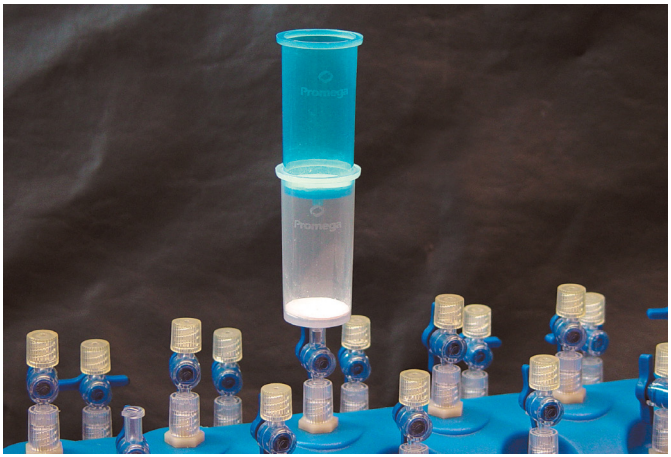

Figure 1. The blue PureYield™ Clearing Column on a white PureYield™ Maxi Binding Column. The two columns sit on a vacuum manifold port.

Note: Perform all purification and elution steps at room temperature.

2. Pour approximately one-half of the lysate into the blue PureYield™ Clearing Column (the column capacity will not allow all of the lysate to be added at once). When processing multiple samples you will need to keep track of which lysate is being placed into which column to avoid cross-contamination.
3. Apply maximum vacuum. The lysate will pass through the clearing membrane in the PureYield™ Clearing Column, and the DNA will bind to the binding membrane in the PureYield™ Maxi Binding Column. Continue the vacuum until all the liquid has passed through both columns.
4. Add the remainder of the lysate to the appropriate clearing column and allow it to pass through both columns as described above.
5. Slowly release the vacuum from the filtration device before proceeding. Remove and discard the PureYield™ Clearing Column, leaving the PureYield™ Maxi Binding Column on the vacuum manifold.

Wash

6. Add 5ml of Endotoxin Removal Wash (as prepared in Section 3) to the PureYield™ Maxi Binding Column, turn on the vacuum and allow the vacuum to pull the solution through the column. For ease of use, the PureYield™ Maxiprep column is labeled with 5, 10, and 20ml fill levels (Figure 2), allowing you to carefully pour or pipette buffers directly into columns.

   Note: If the solution does not pass through the membrane, it could be due to small amounts of particulates. If this occurs, remove the binding column containing the wash solution from the vacuum manifold and place it in a 50ml disposable plastic centrifuge tube. Centrifuge the tube in a swinging bucket rotor for 5 minutes at 2,000 × *g* to allow the wash to pass through the membrane. After centrifugation, remove the binding column and place it back on the vacuum manifold for the next step.

Figure 2. PureYield™ Maxiprep column showing volume labels.

7. Add 20ml of Column Wash (as prepared in Section 3) to the binding column and allow the vacuum to draw the wash through.

8. Dry the membrane by applying a vacuum for 5 minutes. After drying, the tops of the DNA binding membranes should appear dry and there should be no detectable ethanol odor. If the binding column membrane tops appear wet or there is a detectable ethanol odor, repeat the vacuum step to dry the membrane for an additional 5 minutes.

   If more than six samples are being processed at once, the initial drying step should be increased to 10 minutes to compensate for the reduced vacuum.

9. Remove the PureYield™ Maxi Binding Column from the vacuum manifold. Tap the tip of the column on a paper towel to remove excess ethanol. Wipe any excess ethanol from the outside of the tube.

4.B. DNA Purification (continued)

Elute by Vacuum (alternatively, see Elute by Centrifugation, below)

Note: Elution using the Eluator™ Vacuum Elution Device results in better DNA recovery and yield than elution by centrifugation.

10. Place a 1.5ml microcentrifuge tube in the base of the Eluator™ Vacuum Elution Device, securing the tube cap in the open position as shown (Figure 3, Panel A).
11. Assemble the Eluator™ Device and insert the DNA binding column into it, making sure the column is fully seated on the collar.
12. Place the Eluator™ Device assembly, Figure 3, Panel B, onto a vacuum manifold.
13. Add 1ml of Nuclease-Free Water to the binding column. Apply maximum vacuum for 1 minute or until all liquid has passed through the column.
14. Remove the 1.5ml tube and save for DNA quantitation and gel analysis.

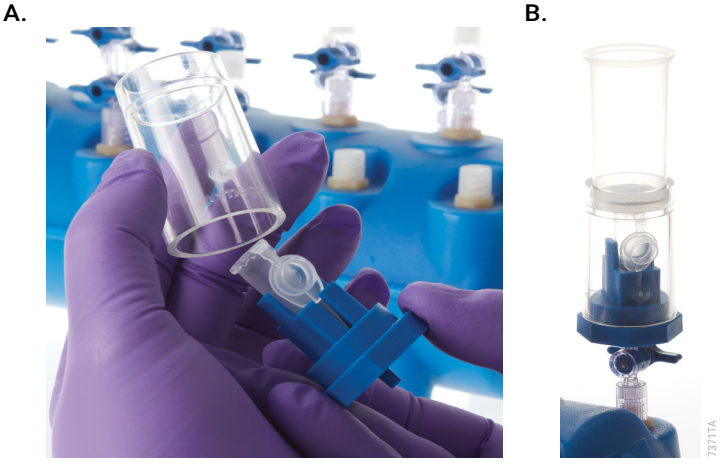

Figure 3. Elution by vacuum. Panel A. A 1.5ml microcentrifuge tube is placed in the base of the Eluator™ Vacuum Elution Device (Cat.# A1071) with the tube cap secured in the open position. **Panel B**. The Eluator™ Vacuum Elution Device assembly, including the binding column, ready for use on a vacuum manifold.

Elute by Centrifugation

Note: Use a room temperature centrifuge with a swinging bucket rotor for the elute by centrifugation steps. To ensure complete passage of solutions through columns, **do not cap** the 50ml tube during centrifugation.

15. To elute the DNA place the PureYield™ Maxi Binding Column into a new 50ml disposable plastic centrifuge tube. Add 1.5ml of Nuclease-Free Water to the DNA binding membrane in the binding column.

    Note: If greater DNA concentration is desired, elute the plasmid in 1ml of water. Yield will be decreased, but concentration will be increased.

16. Centrifuge the PureYield™ Maxi Binding Column in a swinging bucket rotor at 2,000 × *g* for 5 minutes.
17. Collect the eluate from the 50ml tube and transfer to a 1.5ml tube if desired. **Note:** The recovered solution volume may be less than the amount of water added to elute the DNA.

Note: To increase the DNA concentration, perform an ethanol precipitation: Add one-tenth of a volume of 3M sodium acetate (pH 5.2) and 2.5 volumes 95% ethanol. Mix well and place on ice for 15 minutes. Pellet the DNA at 14,000 × *g* for 10 minutes in a microcentrifuge. Discard the supernatant. Wash the pellet with 70% ethanol, mix well and centrifuge at 14,000 × *g* for 2–5 minutes. Discard the ethanol, dry the pellet, then resuspend the DNA in water or TE buffer with pipetting.

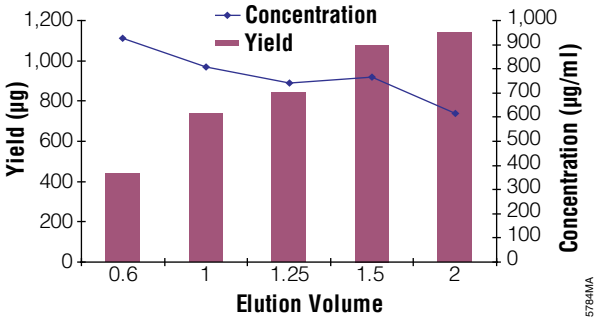

Figure 4. Concentration and yield of DNA from a variety of elution volumes. Cultures (250ml) of JM109 cells containing phMGFP plasmid were grown under standard conditions. Cells were pelleted and DNA purified using the PureYield™ Plasmid Maxiprep System. Elution volumes ranged from 0.6–2ml, when eluted by centrifugation. Each data point is the average of three sample results. Note that the volumes eluted from the column are typically less than the volume added due to rehydration of the membrane.

5. Supplemental Information

5.A. Considerations Regarding the PureYield™ Plasmid Maxiprep System

One of the most important aspects of any plasmid preparation is effective cell lysis. Lysis is usually the limiting step, determining how much cell mass can be processed in a plasmid preparation. If resuspended cells have too great a density, the lysis solution will be less effective in releasing plasmid into the lysate. Exceeding the cell culture volume limit suggested by the kit manufacturer (for PureYield™ Plasmid Maxiprep System the limit is 250ml) can lead to reduced, rather than increased, yields.

It is possible to improve lysis efficiency by increasing the volumes of resuspension, lysis and neutralization solutions used. With the PureYield™ Plasmid Maxiprep System the volumes listed in the protocol (12ml) for each of these solutions will give effective lysis for 250ml of cell culture. Doubling the resuspension, lysis and neutralization solution volumes to 24ml will give efficient lysis for cell culture volumes >250ml and the binding column membrane can support this increased volume of lysate if the cells are lysed sufficiently, allowing more plasmid to be bound and eluted.

5.A. Considerations Regarding the PureYield™ Plasmid Maxiprep System (continued)

For plasmids with a low copy number, BACs and cosmids, binding capacity of the membrane is not a limiting factor; one can load very large amounts of lysate onto the binding column to increase yield. However, for high-copy-number plasmids the maximum amount of culture volume is 250ml; volumes >250ml will contain more plasmid DNA than the binding column can bind.

Optimal resuspension, lysis and neutralization solution volumes will depend on the culture media and cell type used and should be determined by the individual researcher. The PureYield™ Plasmid Maxiprep System provides enough of these solutions for 12ml of each per preparation, which is sufficient for up to 250ml cell culture. The solutions are also available for sale individually (see Section 2 for details) for researchers interested in enhancing the effectiveness and flexibility of their plasmid preparations.

The high-speed centrifugation step (Section 4.A, Step 6) is designed to remove most or all of the cellular debris from the lysate. Some debris may not pellet effectively; in this case, it can be removed using the blue PureYield™ Clearing Column. Excessive mixing after the addition of the Cell Lysis Solution and Cell Neutralization Solution can cause the centrifuged lysate to be cloudy and this cloudiness can cause clogging of the clearing or binding columns. Cloudy lysate should be recentrifuged or filtered through Miracloth™ before loading onto the column.

5.B. Selection and Preparation of Plasmids and *E. coli* Strains

Plasmid DNA can be purified from overnight cultures of *E. coli* with the PureYield™ Plasmid Maxiprep System. The yield of plasmid DNA will vary depending on a number of factors, including the plasmid copy number, cell density of bacterial culture, type of culture medium and the bacterial strain used. Plasmid copy number is an important factor affecting plasmid DNA yield. Copy number is determined primarily by the region of DNA surrounding and including the origin of replication. This region, known as the replicon, controls replication of plasmid DNA by bacterial enzyme complexes. Some DNA sequences, when inserted into a particular plasmid, can lower the copy number of the plasmid by interfering with replication.

Choose a single, well-isolated colony from a fresh Luria-Bertani (LB) agar plate (containing antibiotic) and use the colony to inoculate 1–10ml of LB media (also containing antibiotic). The inoculated medium should be incubated for 8 hours at 37°C to achieve logarithmic growth. This starter culture should then be diluted 1:500 to inoculate a larger volume of culture media containing antibiotic, which is incubated for 12–16 hours at 37°C. An $O.D._{600}$ of 2–4 for high-copy-number plasmids ensures that bacteria have reached the proper growth density for harvesting and plasmid DNA isolation.

5.C. Choosing a Bacterial Strain

Endonuclease I is a 12kDa periplasmic protein that degrades double-stranded DNA. This protein is encoded by the gene *endA*. The *E. coli* genotype endA1 refers to a mutation in the wildtype *endA* gene, which produces an inactive form of the nuclease. *E. coli* strains with this mutation are referred to as *EndA*–. Table 1 contains a list of *EndA*– and *EndA+ E. coli* strains.

Table 1. *EndA*– and *EndA+* Strains of *E. coli*.

EndA–	*EndA+*
BJ5183	BL21(DE3)
DH1	CJ236
DH20	HB101
DH21	JM83
DH5α™	JM101
JM103	JM110
JM105	LE392
JM106	MC1061
JM107	NM522 (all NM series strains are *EndA+*)
JM108	NM554
JM109	P2392
Single Step (KRX) Cells	PR700 (all PR series strains are *EndA+*)
MM294	Q358
SK1590	RR1
SK1592	TB1
SK2267	TG1
SRB	Y1088 (all Y10 series strains are *EndA+*)
TOP10	BMH 71-18
XL1-Blue	ES1301
XLO	

Note: Using the PureYield™ Plasmid Maxiprep System, high-quality DNA is easily obtained from both *EndA+* and *EndA*– bacterial strains.

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5.D. Composition of Buffers and Solutions

Cell Resuspension Solution

50mM	Tris-HCl (pH 7.5)
10mM	EDTA (pH 8.0)
100µg/ml	RNase A

Cell Lysis Solution

0.2M	NaOH
1%	SDS

Neutralization Solution

4.09M	guanidine hydrochloride (pH 4.2)
759mM	potassium acetate
2.12M	glacial acetic acid

Column Wash

162.8mM	potassium acetate
22.6mM	Tris-HCl (pH 7.5)
0.109mM	EDTA (pH 8.0)

Before use add 95% ethanol as directed in Section 3. Final concentrations will be approximately 60% ethanol, 60mM potassium acetate, 8.3mM Tris-HCl and 0.04mM EDTA.

6. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
Poor cell lysis	Too many bacterial cells in culture medium. Use LB medium to grow bacteria. The use of rich medium or excessive culture volumes may lead to a biomass value too high for complete lysis. All media should contain antibiotics at the appropriate concentration.
	Poor resuspension of bacterial cell pellet. The cell pellet must be thoroughly resuspended prior to cell lysis. Pipet or disperse (using an applicator stick) the pellet with Cell Resuspension Solution. No cell clumps should be visible after resuspension.
No plasmid DNA	Ethanol was not added to Column Wash. Prepare the Column Wash as instructed (Section 3) before beginning the procedure.
	Isopropanol was not added to Endotoxin Removal Wash. Prepare the Endotoxin Removal Wash as instructed (Section 3) before beginning the procedure.

Symptoms	Causes and Comments
No plasmid DNA (continued)	PureYield™ Clearing Column clogged due to: • Too much cell mass. Use less biomass or non-rich medium. • Lysate not centrifuged at high enough × *g*. Use 14,000 × *g* for 20 minutes or 7,000 × *g* for 30 minutes. • If lysate is cloudy after centrifugation, filter through Miracloth™.
	PureYield™ Binding Column clogged due to: • Particulates in the lysate. Transfer binding column to 50ml centrifuge tube and centrifuge in a swinging bucket rotor at room temperature for 5 minutes at 2,000 × *g*. • Insufficient vacuum. Ensure that vacuum source is at a minimum of 650mm Hg.
Lysate is cloudy	SDS precipitation may have occurred: • Centrifuge at room temperature to clear lysate. • If debris not sufficiently removed by centrifugation, filter lysate through Miracloth™ before adding lysate to column.
Denaturation of plasmid DNA	Overincubation during lysis step, Section 4.A. Total incubation of cell suspension with Cell Lysis Solution should not exceed 5 minutes.
Genomic DNA contamination	Vortexing or overmixing after addition of the Cell Lysis Solution. Do not vortex samples after addition of Cell Lysis Solution to prevent shearing of genomic DNA.
Low plasmid DNA yields	Overgrowth of bacterial culture by nontransformed bacteria: • Make certain that antibiotics were used in all media, liquid and solid. Do not culture bacteria longer than 24 hours. Optimal culture length is 12–16 hours. • Bacterial culture is too old. Inoculate antibiotic-containing media with freshly isolated bacterial colony from an overnight plate.
	Some bacterial cells are more resistant to lysis than others and may require incubation for up to 5 minutes for efficient lysis. The lysate may not clear completely, but do not extend lysis beyond 5 minutes as lysis for >5 minutes may result in nicked or single-stranded DNA.
	Wrong reagents used. Make certain that Column Wash is diluted with ethanol and the Endotoxin Removal Wash is diluted with isopropanol before use (Section 3). Use only the reagents supplied with the PureYield™ Plasmid Maxiprep System.

6. Troubleshooting (continued)

Symptoms	Causes and Comments
Low plasmid DNA yields (continued)	Plasmid DNA yield not accurately quantitated. Quantitation by absorbance at A_{260} may overestimate yield due to absorbance by a variety of contaminants such as RNA and protein. This is especially true for low-copy-number plasmids when the ratio of contaminants to plasmid DNA is high. Use agarose gel/ethidium bromide quantitation.
	For plasmids larger than 10kb, yield may be increased by heating the water to 65°C at the elution step. Add water to the binding column and let sit for 1 minute. Elute as normal.
Ethanol carryover	Ethanol carryover is detected in the final product. Dry the column for an additional 5 minutes on the vacuum manifold.
	Column Wash could be present on the outside of the column due to splashing during the wash step. Remove any residual ethanol from the outside of the column prior to elution, as directed in Section 4.B, Step 9.

7. Using the Vac-Man® Laboratory Vacuum Manifold

7.A. Description

The Vac-Man® Laboratory Vacuum Manifold (Figure 5), when used in conjunction with any of the Wizard®, PureYield™ or SV Isolation Systems, is the ideal system for rapid, effective nucleic acid purification. Reliable, sturdy and easy to use, the Vac-Man® Laboratory Vacuum Manifold will process from 1 to 20 samples simultaneously. Because each manifold comes complete with a set of 20 individually controlled One-Way Luer-Lok® Stopcocks, as many or as few samples as desired can be processed at one time. The One-Way Luer-Lok® Stopcocks are designed to accommodate Promega Minicolumns and Maxi/Megacolumns, as well as the PureYield™ Clearing Columns and PureYield™ Maxi Binding Columns.

7.B. Setup and Operation

The Vac-Man® Laboratory Vacuum Manifold is designed for use with a small benchtop laboratory vacuum source (pump). The manifold is **not** designed for use under a high-pressure vacuum (see label for further information).

Note: If you have any questions about the suitability of your vacuum source, please contact your local Promega branch office or distributor. Contact information available at: **www.promega.com** or e-mail: **techserv@promega.com**

Do not substitute chemicals. Always follow safe laboratory practices, including the use of safety glasses and a laboratory coat.

1. Inspect the manifold connectors, One-Way Luer-Lok® Stopcocks and Spinlock II Adapters, included with the manifold, for any evidence of wear or mishandling. Do not use the manifold if significant damage, such as a crack or an abrasion, is apparent.

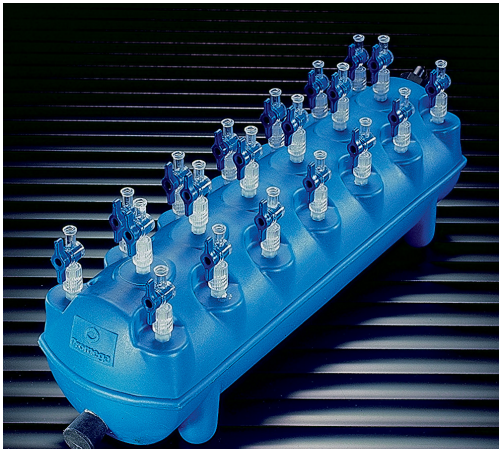

Figure 5. The Vac-Man® Laboratory Vacuum Manifold (Cat.# A7231).

2. Insert the Neoprene Stopper into the hole located at one end of the manifold. The stopper should fit snugly. The Neoprene Stopper functions as a safety valve in the event of excessive pressure buildup.

   Note: Do not restrict the removal of the Neoprene Stopper in any way (e.g., with tape, wire, etc.).

3. Attach a One-Way Luer-Lok® Stopcock to each of the 20 connectors located on the top of the manifold. Secure each stopcock by turning the stopcock collar about one-quarter turn clockwise. Do not force the collar; this may strip the Luer-Lok® connector threads on the manifold. Close all the stopcocks by turning each stopcock handle to a horizontal position.

4. **Optional:** The Spinlock II Adapters act as an extension of the Luer-Lok® Stopcocks. These Adapters may be used to make manipulation of the stopcock valves easier.

   Insert a Spinlock II Adapter into a One-Way Luer-Lok® Stopcock. Secure by turning about one-quarter turn clockwise. Do not force as this may strip the connector threads.

5. Attach a vacuum hose to the hose connector located at the end of the manifold opposite the rubber stopper hole. Like the stopper, the hose connection can function as a safety valve in the event of inadvertent excess pressure buildup.

   Note: Do not fix the vacuum hose to the manifold with a hose clamp.

6. Connect the vacuum hose to your vacuum source. The Vac-Man® Laboratory Vacuum Manifold is now ready for use.

   For additional information on using and cleaning the Vac-Man® Laboratory Vacuum Manifold, please visit: **www.promega.com/protocols/**

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8. Related Products

Product	Size	Cat.#
Cell Resuspension Solution (CRA)	315ml	A7115
Cell Lysis Solution (CLA)	315ml	A7125
Neutralization Solution (NSB)	500ml	A1485
Eluator™ Vacuum Elution Device*	4 each	A1071
Vac-Man® Laboratory Vacuum Manifold, 20-sample capacity	each	A7231
Vac-Man® Jr. Laboratory Vacuum Manifold, #8	each	A7660
Vac-Man® Jr. Laboratory Vacuum Manifold, #10	each	A3110
PureYield™ Plasmid Midiprep System	25 preps	A2492
	100 preps	A2495
	300 preps	A2496
PureYield™ Plasmid Miniprep System	100 preps	A1223
	250 preps	A1222
TNT® T7 Quick Coupled Transcription/Translation System*	40 reactions	L1170
TNT® SP6 Coupled Reticulocyte Lysate System*	40 reactions	L4600
TNT® T7 Coupled Reticulocyte Lysate System*	40 reactions	L4610
TNT® T3 Coupled Reticulocyte Lysate System*	40 reactions	L4950
TNT® T7/T3 Coupled Reticulocyte Lysate System*	40 reactions	L5010
TNT® T7/SP6 Coupled Reticulocyte Lysate System*	40 reactions	L5020
TransFast™ Transfection Reagent	1.2mg	E2431
GoTaq® Green Master Mix*†	100 reactions	M7122
	1,000 reactions	M7123
GoTaq® DNA Polymerase*†	100u	M3001
	500u	M3005
	2,500u	M3008
PCR Master Mix*	100 reactions	M7502
	1,000 reactions	M7505

*For Laboratory Use.

†Different Cat.# may apply for customers in Europe. Visit: **www.promega.com/products/pcr/** for the amplification product catalog numbers appropriate for your location.

9. Summary of Changes

The following changes were made to the 9/23 revision of this document:

1. Updated Section 8 and patent statements.
2. Changed font and cover image.
3. Made minor text edits.

[a]Patents Pending.