Technical Manual

PureYield™ Plasmid Midiprep System

INSTRUCTIONS FOR USE OF PRODUCTS A2490, A2492, A2495 AND A2496.

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PureYield™ Plasmid Midiprep System

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Please visit the web site to verify that you are using the most current version of this Technical Manual. Please contact Promega Technical Services if you have questions on use of this system. E-mail: techserv@promega.com

1. Description

As research moves from DNA sequencing to analysis of gene function, the need has increased for rapid methods by which to isolate large quantities of high-quality plasmid DNA. The PureYield™ Plasmid Midiprep System[a] is designed to isolate high-quality plasmid DNA for use in eukaryotic transfection and in vitro expression experiments. The system provides a rapid method for purification using a silica-membrane column. Plasmid DNA can be purified in less than 45 minutes, greatly reducing the time spent on purification compared to silica resin or other membrane column methods.

The PureYield™ Plasmid Midiprep System also incorporates a unique Endotoxin Removal Wash, designed to remove substantial amounts of protein, RNA and endotoxin contaminants from purified plasmid DNA, improving the robustness of sensitive applications such as eukaryotic transfection, in vitro transcription and coupled in vitro transcription/translation. Purification is achieved without isopropanol precipitation of purified plasmid DNA or extensive centrifugation, providing rapid purification as well as a high concentration of pure plasmid DNA from a single method.

The PureYield™ Plasmid Midiprep System is designed to purify 100–200µg of plasmid DNA with an A_{260}/A_{280} >1.7 from a 50–100ml overnight culture of bacteria, transformed with a high copy number plasmid, with a total biomass ($O.D._{600}$ of culture × volume of culture) of 100–200. Larger amounts of biomass (e.g., up to 250ml of bacterial culture) can be processed with additional volumes of solutions.

The PureYield™ System is designed to clear lysate with a fixed-angle centrifugation step, followed by binding and washing using vacuum. Alternative protocols are available using all centrifugation (with a swinging bucket rotor), vacuum or a combination (see Section 5, Supplemental Information). A tabletop centrifuge is required for elution by centrifugation. Elution can also be accomplished by vacuum (Section 4), using the Eluator™ Vacuum Elution Device (Cat.# A1071). Vacuum elution results in the best DNA recovery and yield.

The PureYield™ Systems (Cat.# A2490, A2492, A2495, A2496) contain sufficient reagents for 4 × 50ml preps, 25 × 50ml preps and 100 × 50ml preps and 300 × 50ml preps, respectively. When working with low copy number plasmids, larger culture volumes (e.g., 101–250ml) or very dense cultures ($O.D._{600}$ 4–5 O.D./ml), it may be beneficial to increase the volumes of Cell Resuspension Solution (CRA), Cell Lysis Solution (CLA) and Neutralization Solution (NSB) to increase the efficiency of alkaline lysis and yield. If additional buffers are needed, their compositions are provided in Section 5.F. Alternatively, buffers may be purchased separately (Cat.# A7115, A7125, A1485; see Section 2 or 7).

2. Product Components and Storage Conditions

Product	Size	Cat.#
PureYield™ Plasmid Midiprep System	4 preps	A2490

Each system contains sufficient reagents for 4 × 50 ml preps. Includes:

- 12ml Cell Resuspension Solution (CRA)
- 12ml Cell Lysis Solution (CLA)
- 20ml Neutralization Solution (NSB)
- 15ml Endotoxin Removal Wash
- 33ml Column Wash
- 13ml Nuclease-Free Water
- 4 each PureYield™ Clearing Columns
- 4 each PureYield™ Binding Columns

Product	Size	Cat.#
PureYield™ Plasmid Midiprep System	25 preps	A2492

Each system contains sufficient reagents for 25 × 50 ml preps. Includes:

- 75ml Cell Resuspension Solution (CRA)
- 75ml Cell Lysis Solution (CLA)
- 130ml Neutralization Solution (NSB)
- 85.3ml Endotoxin Removal Wash
- 210ml Column Wash
- 25ml Nuclease-Free Water
- 25 each PureYield™ Clearing Columns
- 25 each PureYield™ Binding Columns

Product	Size	Cat.#
PureYield™ Plasmid Midiprep System	100 preps	A2495

Each system contains sufficient reagents for 100 × 50 ml preps. Includes:

- 315ml Cell Resuspension Solution (CRA)
- 315ml Cell Lysis Solution (CLA)
- 500ml Neutralization Solution (NSB)
- 315ml Endotoxin Removal Wash
- 2 × 381ml Column Wash
- 4 × 25ml Nuclease-Free Water
- 100 each PureYield™ Clearing Columns
- 100 each PureYield™ Binding Columns

Product	Size	Cat.#
PureYield™ Plasmid Midiprep System	300 preps	A2496

Each system contains sufficient reagents for 300 × 50 ml preps. Includes:

- 3 × 315ml Cell Resuspension Solution (CRA)
- 3 × 315ml Cell Lysis Solution (CLA)
- 3 × 500ml Neutralization Solution (NSB)
- 3 × 315ml Endotoxin Removal Wash
- 6 × 381ml Column Wash
- 2 × 150ml Nuclease-Free Water
- 300 each PureYield™ Clearing Columns
- 300 each PureYield™ Binding Columns

Storage Conditions: Store all system components at room temperature (22–25°C).

Larger culture volumes (101–250ml) will require greater volumes of solutions than provided with the PureYield™ Plasmid Midiprep Systems. The solution compositions are provided in Section 5.F or can be purchased separately (Cat.# A7115, A7125, A1485).

2. Product Components and Storage Conditions (continued)

Items Available Separately

Product	Size	Cat.#
Cell Lysis Solution (CLA)	315 ml	A7125
Cell Resuspension Solution (CRA)	315 ml	A7115
Eluator™ Vacuum Elution Device	each	A1071
Neutralization Solution (NSB)	500 ml	A1485

3. Equipment, Supplies and Preparation of Solutions

Materials to Be Supplied by the User

- isopropanol
- ethanol, 95%
- tabletop centrifuge
- swinging bucket rotor
- 50ml disposable plastic screw-cap tubes (e.g., Corning or Falcon™ brand)
- high-speed centrifuge capable of at least 15,000 × *g* and appropriate tubes
- vacuum pump, single- or double-stage, producing pressure of approximately 650mm Hg (25.6 inches Hg); see table for comparison of inches of Hg to other pressure measurements. Contact your Promega representative for more information about vacuum pumps.
- vacuum manifold (e.g., Vac-Man® Laboratory Vacuum Manifold [Cat.# A7231])
- **optional:** Eluator™ Vacuum Elution Device (Cat.# A1071)

Comparison of Inches of Hg to Other Pressure Measurements for the PureYield™ System.

25.6 Inches Hg
86.7kPa
50 Torr
0.855atm
12.57psi
65.0cm Hg
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

- **4 preps system (Cat.# A2490):**
  Add 10ml of isopropanol to the Endotoxin Removal Wash bottle.
- **25 preps system (Cat.# A2492):**
  Add 57ml of isopropanol to the Endotoxin Removal Wash bottle.
- **100 preps system (Cat.# A2495):**
  Add 210ml of isopropanol to the Endotoxin Removal Wash bottle.
- **300 preps system (Cat.# A2496):**
  Add 210ml of isopropanol to **each** bottle of Endotoxin Removal Wash.

Column Wash

- **4 preps system (Cat.# A2490):**
  Add 55ml of 95% ethanol to the Column Wash bottle.
- **25 preps system (Cat.# A2492):**
  Add 350ml of 95% ethanol to the Column Wash bottle.
- **100 preps system (Cat.# A2495):**
  Add 635ml of 95% ethanol to **each** Column Wash bottle.
- **300 preps system (Cat.# A2496):**
  Add 635ml of 95% ethanol to **each** Column Wash bottle.

4. Standard DNA Purification Protocol

This section details our standard protocol for lysis of bacterial cell cultures and DNA purification. This protocol is robust, fast and easy to use.

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 purification and elution steps at room temperature.

1. Grow 50–250ml of transformed *E. coli* bacterial cell culture overnight (16–21 hours) at optimal culture conditions.

   Note: This protocol is optimized for 50–250ml of culture at an $O.D._{600}$ = 2–4.

2. Pellet the cells using centrifugation at 5,000 × *g* for 10 minutes and discard supernatant. Drain tubes on a paper towel to remove excess media.

   Table 1. Solution Volumes Required to Generate Lysate.

	Bacterial Culture Volume	
Solution Name	**50–100ml**	**101–250ml**
Cell Resuspension Solution	3ml	6ml*
Cell Lysis Solution	3ml	6ml*
Neutralization Solution	5ml	10ml*

*Additional solutions will need to be purchased or made for processing 101–250ml culture volumes.

3. Resuspend the cell pellets in Cell Resuspension Solution.

4. Standard DNA Purification Protocol (continued)

4. Add Cell Lysis Solution and mix by gently inverting the tube 3–5 times or mix lysate by gently rolling the tube. Incubate for 3 minutes at room temperature.

   Note: If the Cell Lysis Solution becomes too cold, SDS precipitation may occur, leading to poor cell lysis. If a precipitate has formed, warm the Cell Lysis Solution to 37°C with gentle shaking.

5. Add Neutralization Solution to the lysed cells, cap and mix by gently inverting the tube 5–10 times.

6. Centrifuge the lysate at room temperature, 15,000 × *g* for 15 minutes. This centrifugation will pellet the bulk of the cellular debris. Remaining debris is removed using the PureYield™ Clearing Columns.

To differentiate the PureYield™ Clearing and PureYield™ Binding columns, note that the **clearing columns are blue**, while the **binding columns are white**.

7. Assemble a column stack by nesting a PureYield™ Clearing Column (**blue**) into the top of a PureYield™ Binding Column (**white**). Place the assembled column stack onto the vacuum manifold as shown in Figure 1.

8. Decant the cleared lysate into the PureYield™ Clearing Column. Do **not** allow the pelleted debris to fall into the column.

9. Apply 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™ Binding Column. Continue the vacuum until all the liquid has passed through both columns.

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

10. **Slowly release** the vacuum from the filtration device before proceeding. Remove the PureYield™ Clearing Column, leaving the PureYield™ Binding Column on the vacuum manifold.

    Note: If the vacuum is released too quickly, the membrane may detach from the column. If the binding membrane becomes detached, tap it down gently with a gloved finger or the large end of a sterile 1.0ml pipette tip, or turn on the vacuum and allow the pressure to reseat the membrane.

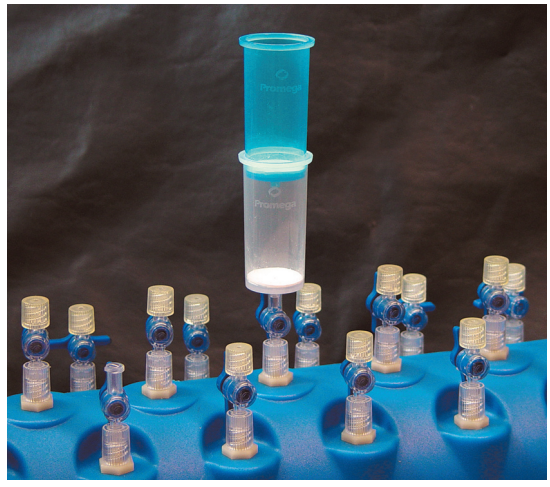

Figure 1. The blue clearing column inserted into the white binding column. The two columns sit on a vacuum manifold port.

Wash

11. Add 5.0ml of Endotoxin Removal Wash to the column, and allow the vacuum to pull the solution through the column. For ease of use, the PureYield™ Midiprep Column is labeled with 5, 10 and 20ml fill levels (Figure 2). Buffers can be pipetted or carefully poured to the appropriate volume.

Figure 2. PureYield™ Midiprep Columns are labeld with 5, 10 and 20ml fill levels.

4. Standard DNA Purification Protocol (continued)

12. Add 20ml of Column Wash Solution to the column, and allow the vacuum to draw the solution through.

13. Dry the membrane by applying a vacuum for 30 seconds to 1 minute. After drying, the tops of the DNA binding membranes should appear dry and there should be no detectable ethanol odor.

If the DNA binding membrane tops appear wet or there is a detectable ethanol odor, **repeat** the vacuum dry step for an additional 30 seconds.

14. Remove the PureYield™ Binding Column from the vacuum manifold. Tap the tip of the column on a paper towel to remove excess ethanol. Place the column into a new 50ml disposable plastic tube (e.g., Corning or Falcon™).

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

    Note: Elution by vacuum using the Eluator™ Vacuum Elution Device (Cat.# A1071) results in better DNA recovery and yield than elution by centrifugation.

15. 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 2, Panel A).

16. Assemble the Eluator™ Device and insert the DNA binding column into it, making sure the column is fully seated on the collar.

17. Place the Eluator™ Device assembly onto a vacuum manifold (Figure 2, Panel B).

18. Add 400–600µl of Nuclease-Free Water to the DNA binding column. Apply maximum vacuum for 1 minute or until all liquid has passed through the column.

    Note: Elution volume will affect both concentration and yield. When using the Eluator™ Vacuum Elution Device, an elution volume of 400µl will give a higher concentration but lower yield than using 600µl. If yield is more important than concentration, increase the elution volume to 600µl. Elution volume should not be less than 400µl or DNA recovery will be poor.

19. Remove the tube and save for DNA quantitation and gel analysis.

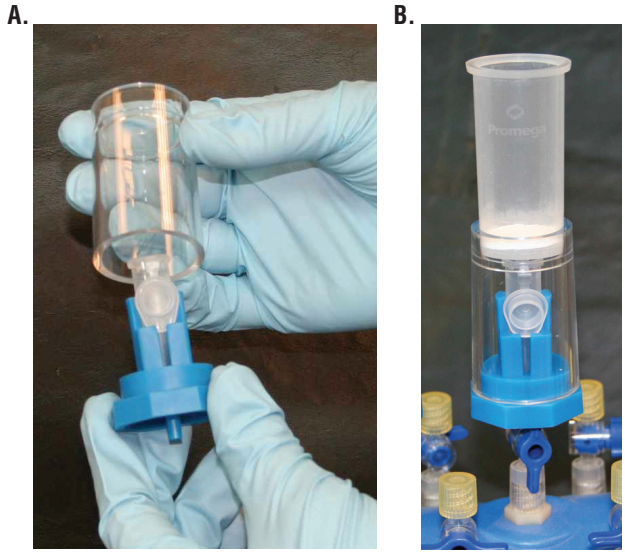

Figure 3. Elution by vacuum. Panel A. A 1.5ml microcentrifuge tube is placed in the base of the Eluator™ Vacuum Elution Device and the microcentrifuge tube cap is locked as shown. **Panel B**. The final Eluator™ Vacuum Elution Device assembly, including the binding column, ready for use on a vacuum manifold.

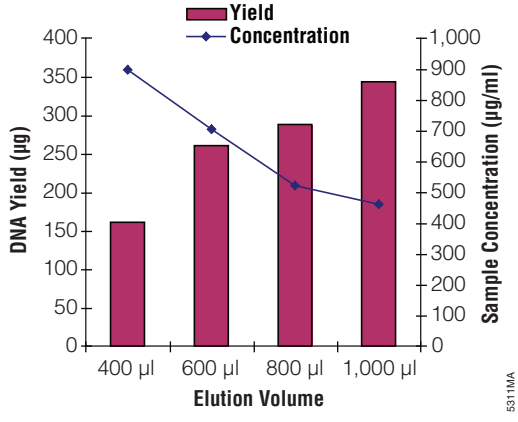

Figure 4. Concentration and yield of phMGFP purified from varying elution volumes. Fifty-milliliter cultures of JM109 cells containing phMGFP were grown under standard conditions. Cells were pelleted, and DNA was purified using the PureYield™ Plasmid Midiprep System (vacuum method). Elution volume varied from 400µl to 1,000µl. After eluting with a spin of 1,500 × g for 5 minutes, elution volume, DNA concentration and yield were determined. Each data point is the average of three samples. Note that the volumes eluted from the column are typically less than the volume added due to rehydration of the binding membrane.

4. Standard DNA Purification Protocol (continued)

Elute by Centrifugation

Use **only** a **centrifuge with a swinging bucket rotor** for elution by centrifugation. To ensure complete passage of solutions through columns, do not cap the 50ml tube during centrifugation.

20. To elute the DNA, add 600µl of Nuclease-Free Water to the DNA binding membrane in the PureYield™ Binding Column.

    Note: Elution volume will affect both concentration and yield. Use a 600–800µl elution volume. Using a 600µl elution volume will give a higher concentration but lower yield than using an 800µl elution volume. If yield is more important than concentration, increase the elution volume to 800µl. Elution volume should not be less than 400µl or DNA recovery will be poor. Additional DNA may be obtained with a second elution, although purity may be affected. Total volume recovered from both elutions should not exceed 1.0ml. Volumes eluted from the columns are typically less (25–50%) than the volume added due to rehydration of the membrane.

21. Centrifuge the PureYield™ Binding Column at 1,500–2,000 × *g* for 5 minutes.

22. Collect the filtrate from the 50ml tube and transfer to a 1.5ml tube if desired.

    Note: If a higher concentration is desired for subsequent applications, perform an ethanol precipitation (see Section 6 for protocol).

5. Supplemental Information

Alternative protocols can be more sensitive to the biomass of the culture particularly with large volumes or rich media. Optimization may be required in these cases.

Table 2. Choosing an Alternative Plasmid DNA Purification Protocol.

Protocol	Benefits
Centrifugation (Section V.A)	Eliminates use of a vacuum.
Vacuum (Section V.B)	Faster sample processing; eliminates need for centrifugation.
Quick Combination Method (Section V.C)	Combines efficient lysate clearing by centrifugation with rapid washing and binding done by vacuum.

5.A. DNA Purification by Centrifugation

Note: Perform all purification steps at room temperature.

1. Grow 50–100ml of transformed *E. coli* bacterial cell culture overnight (16–21 hours) at optimal culture conditions.
2. Pellet the cells using centrifugation at 5,000 × *g* for 10 minutes and discard supernatant. Drain tubes on a paper towel to remove excess media.

Table 3. Volumes of Solutions Required to Create Lysate.

	Bacterial Culture Volume	
Solution Name	**<50ml**	**50–100ml**
Cell Resuspension Solution	2ml	3ml
Cell Lysis Solution	2ml	3ml
Neutralization Solution	3.3ml	5ml

3. Resuspend the cell pellets in Cell Resuspension Solution.
4. Add Cell Lysis Solution and mix by gently inverting the tube 3–5 times or mix lysate by gently rolling the tube. Incubate for 3 minutes at room temperature.

   Note: If the Cell Lysis Solution becomes too cold, SDS precipitation may occur, leading to poor cell lysis. If a precipitate has formed, warm the Cell Lysis Solution to 37°C with gentle shaking.
5. Add Neutralization Solution to the lysed cells, cap and mix by gently inverting the tube 3–5 times. Allow the lysate to sit for 2–3 minutes in an upright position to allow a white flocculent precipitate to form. **It is important** to wait for the precipitate to form to ensure thorough lysate clearing.

Use **only** a room temperature **centrifuge with a swinging bucket rotor** for ***all*** centrifugation steps in this protocol. To ensure complete passage of solutions through columns, do not cap the 50ml tube during centrifugation.

To differentiate the PureYield™ Clearing and PureYield™ Binding columns, note that the **clearing columns are blue**, while the **binding columns are white.**

6. Place a PureYield™ Clearing Column (**blue**) into a new 50ml disposable plastic tube (e.g., Corning or Falcon™).
7. Pour the lysate into the PureYield™ Clearing Column. Incubate for 2 minutes to allow the cellular debris to rise to the top.

   Note: Failure to perform the incubation may lead to incomplete lysate filtration.

5.A. DNA Purification by Centrifugation (continued)

8. Centrifuge the PureYield™ Clearing Column in a tabletop centrifuge at 1,500 × *g* for 5 minutes. If not all the lysate has filtered through, repeat the centrifugation at 1,500 × *g* for another 5 minutes. A small amount of liquid (≤1ml) may remain trapped in the residual insoluble material and will have a minimal effect on results.

   Note: The PureYield™ Clearing Column can be centrifuged at higher speeds if desired (up to 3,000 × *g*).

9. Place a PureYield™ Binding Column (**white**) into a new 50ml disposable plastic tube (e.g., Corning or Falcon™).

10. Pour the filtered lysate into the PureYield™ Binding Column. Centrifuge the column in a tabletop centrifuge at 1,500 × *g* for 3 minutes.

    Wash

11. Add 5.0ml of Endotoxin Removal Wash solution (with isopropanol added; see Section 3) to the PureYield™ Binding Column. For ease of use, the PureYield™ Midiprep Column is labeled with 5, 10 and 20ml fill levels (Figure 2). Buffers can be pipetted or carefully poured to the appropriate volume. Centrifuge at 1,500 × *g* for 3 minutes. Remove the assembly from the centrifuge, and discard the flowthrough. Reinsert the column into the tube.

    Note: Endotoxin Removal Wash can greatly reduce contaminants such as endotoxin, protein, RNA and endonucleases.

12. Add 20ml of Column Wash Solution (with ethanol added; see Section 3) to the PureYield™ Binding Column and centrifuge at 1,500 × *g* for 5 minutes. Remove the assembly from the centrifuge, and discard the flowthrough. Reinsert the column into the tube. Centrifuge at 1,500 × *g* for an additional 10 minutes to ensure the removal of ethanol.

13. Tap the tip of the column on a paper towel to ensure the removal of ethanol from the column. Wipe any excess ethanol from the outside of the tube.

    Elute

14. To elute the DNA, place the binding column in a new 50ml disposable plastic tube (e.g., Corning or Falcon™), and add 600µl Nuclease-Free Water to the DNA binding membrane in the PureYield™ Binding Column.

    Note: Elution volume will affect both yield and concentration (see Figure 3). The recommended elution volume, 600µl, was chosen to optimize the tradeoff between yield and concentration. If yield is more important than concentration, increase the elution volume. If concentration is more important than yield, decrease the elution volume. Elution volume should not be less than 400µl or plasmid recovery will be poor. Additional plasmid may be obtained with a second elution, although purity may be affected.

Total volume recovered from both elutions should not exceed 1.0ml. Volumes eluted from the columns are typically less (25–50%) than the volume added due to rehydration of the membrane.

15. Centrifuge the PureYield™ Binding Column at 1,500–2,000 × *g* for 5 minutes.

16. Collect the filtrate from the 50ml tube and transfer to a 1.5ml tube if desired.

    Note: If a higher concentration is desired for subsequent applications, perform an ethanol precipitation (see Section 6 for protocol).

5.B. DNA Purification by Vacuum

Note: Perform all purification steps at room temperature.

1. Grow 50–100ml of transformed *E. coli* bacterial cell culture overnight (16–21 hours) at optimal culture conditions.

2. Pellet the cells using centrifugation at 5,000 × *g* for 10 minutes and discard supernatant. Drain tubes on a paper towel to remove excess media.

Table 4. Volumes of Solutions Required to Create Lysate.

	Bacterial Culture Volume		
Solution Name	**<50ml**	**50–100ml**	**101–250ml**
Cell Resuspension Solution	2ml	3ml	6ml*
Cell Lysis Solution	2ml	3ml	6ml*
Neutralization Solution	3.3ml	5ml	10ml*

*Additional solutions will need to be purchased or made for processing 101–250ml culture volumes.

3. Resuspend the cell pellets in Cell Resuspension Solution.

   Note: Make sure that cell resuspension is complete.

4. Add Cell Lysis Solution and mix by gently inverting the tube 3–5 times or mix lysate by gently rolling the tube. Incubate for 3 minutes at room temperature.

   Note: If the Cell Lysis Solution becomes too cold, SDS precipitation may occur, leading to poor cell lysis. If a precipitate has formed, warm the Cell Lysis Solution to 37°C with gentle shaking.

5.B. DNA Purification by Vacuum (continued)

5. Add Neutralization Solution to the lysed cells, cap and mix by gently inverting the tube 3–5 times. Allow the lysate to sit for 2–3 minutes in an upright position to allow a white flocculent precipitate to form. **It is important** to wait for the precipitate to form to ensure thorough lysate clearing.

To differentiate the PureYield™ Clearing and PureYield™ Binding columns, note that the **clearing columns are blue**, while the **binding columns are white**.

6. Assemble a column stack by nesting a PureYield™ Clearing Column (**blue**) into the top of a PureYield™ Binding Column (**white**). Place the assembled column stack onto the vacuum manifold as shown in Figure 1.

7. Pour the lysate into the PureYield™ Clearing Column, and incubate for 2–3 minutes to allow the cellular debris to rise to the top.

   Note: Failure to perform the incubation may lead to incomplete lysate filtration.

8. Apply 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™ Binding Column. Continue the vacuum until all the liquid has passed through both columns.

   Note: If some of the liquid does not pass through the PureYield™ Clearing Column, which sometimes happens when dealing with dense cell cultures, remove the PureYield™ Clearing Column, place it in a new 50ml tube and centrifuge at 1,500 × *g* for 5 minutes. Then add the resulting cleared lysate directly to the PureYield™ Binding Column.

9. **Slowly release** the vacuum from the filtration device before proceeding. Remove the PureYield™ Clearing Column, leaving the PureYield™ Binding Column on the vacuum manifold.

   Note: If the vacuum is released too quickly, the membrane may detach from the column. If the binding membrane becomes detached, tap it down gently with a gloved finger or the large end of a sterile 1.0ml pipette tip.

   Wash

10. Add 5.0ml of Endotoxin Removal Wash to the column and allow the vacuum to pull the solution through the column. For ease of use, the PureYield™ Midiprep Column is labeled with 5, 10 and 20ml fill levels (Figure 2). Buffers can be pipetted or carefully poured to the appropriate volume.

    Note: Endotoxin Removal Wash can greatly reduce contaminants such as endotoxin, protein, RNA and endonucleases.

11. Add 20ml of Column Wash Solution to the column and allow the vacuum to draw the solution through.

12. Dry the membrane by applying a vacuum for 30 seconds. After drying, the tops of the DNA binding membranes should appear dry and there should be no detectable ethanol odor.

If the DNA binding membrane tops appear wet or there is a detectable ethanol odor, **repeat** the vacuum dry step for an additional 30 seconds.

13. Remove the PureYield™ 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. Place the column into a new 50ml disposable plastic tube (e.g., Corning or Falcon™).

Elute

Note: An alternative Elute by Vacuum protocol is available in Section 4. This protocol requires an Eluator™ Vacuum Elution Device (Cat.# A1071).

Use **only** a **centrifuge with a swinging bucket rotor** for the elution step in this protocol. To ensure complete passage of solutions through columns, do not cap the 50ml tube during centrifugation.

14. To elute the DNA, place the binding column in a new 50ml disposable plastic tube (e.g., Corning or Falcon™), and add 600µl Nuclease-Free Water to the DNA binding membrane in the PureYield™ Binding Column.

    Note: Elution volume will affect both yield and concentration. The recommended elution volume, 600µl, was chosen to optimize the tradeoff between yield and concentration. If yield is more important than concentration, increase the elution volume. If concentration is more important than yield, decrease the elution volume. Elution volume should not be less than 400µl or plasmid recovery will be poor. Additional plasmid may be obtained with a second elution, although purity may be affected. Total volume recovered from both elutions should not exceed 1.0ml. Volumes eluted from the columns are typically less (25–50%) than the volume added due to rehydration of the membrane.

15. Centrifuge the PureYield™ Binding Column at 1,500–2,000 × *g* for 5 minutes.

16. Collect the filtrate from the 50ml tube and transfer to a 1.5ml tube if desired. **Note:** If a higher concentration is desired for subsequent applications, perform an ethanol precipitation (see Section 6 for protocol).

5.C. DNA Purification by Quick Combination Method

Note: Perform all purification steps at room temperature.

1. Grow 50–250ml of transformed *E. coli* bacterial cell culture overnight (16–21 hours) at optimal culture conditions.
2. Pellet the cells using centrifugation at 5,000 × *g* for 10 minutes and discard supernatant. Drain tubes on a paper towel to remove excess media.

Table 5. Volumes of Solutions Required to Create Lysate.

	Bacterial Culture Volume		
Solution Name	**<50ml**	**50–100ml**	**101–250ml**
Cell Resuspension Solution	2ml	3ml	6ml*
Cell Lysis Solution	2ml	3ml	6ml*
Neutralization Solution	3.3ml	5ml	10ml*

*Additional solutions will need to be purchased or made for processing 101–250ml culture volumes.

3. Resuspend the cell pellets in Cell Resuspension Solution.
4. Add Cell Lysis Solution and mix by gently inverting the tube 3–5 times or mix lysate by gently rolling the tube. Incubate for 3 minutes at room temperature.

   Note: If the Cell Lysis Solution becomes too cold, SDS precipitation may occur, leading to poor cell lysis. If a precipitate has formed, warm the Cell Lysis Solution to 37°C with gentle shaking.
5. Add Neutralization Solution to the lysed cells, cap and mix by gently inverting the tube 3–5 times. Allow the lysate to sit for 2–3 minutes in an upright position to allow a white flocculent precipitate to form. **It is important** to wait for the precipitate to form to ensure thorough lysate clearing.

Use **only** a room temperature **centrifuge with a swinging bucket rotor** for ***all*** centrifugation steps in this protocol. To ensure complete passage of solutions through columns, do not cap the 50ml tube during centrifugation.

To differentiate the PureYield™ Clearing and PureYield™ Binding columns, note that the **clearing columns are blue**, while the **binding columns are white.**

6. Place a PureYield™ Clearing Column (**blue**) into a new 50ml disposable plastic tube (e.g., Corning or Falcon™).

7. Pour the lysate into the PureYield™ Clearing Column. Incubate for 2 minutes to allow the cellular debris to rise to the top.

   Note: Failure to perform the incubation may lead to incomplete lysate filtration.

8. Centrifuge the PureYield™ Clearing Column in a tabletop centrifuge at 1,500 × *g* for 5 minutes. If not all the lysate has filtered through, repeat the centrifugation at 1,500 × *g* for another 5 minutes.

   Note: The PureYield™ Clearing Column can be centrifuged faster if desired (up to 3,000 × *g*).

9. Place a PureYield™ Binding Column (**white**) onto the vacuum manifold.

10. Pour lysate into the PureYield™ Binding Column and apply vacuum. The lysate will pass through the binding column. Continue the vacuum until all the liquid has passed through the PureYield™ Binding Column.

11. **Slowly release** the vacuum from the column before proceeding.

    Note: If the vacuum is released too quickly, the membrane may detach from the column. If the binding membrane becomes detached, tap it down gently with a gloved finger or the large end of a sterile 1.0ml pipette tip.

    Wash

12. Add 5.0ml of Endotoxin Removal Wash to the column and allow the vacuum to pull the solution through. For ease of use, the PureYield™ Midiprep Column is labeled with 5, 10 and 20ml fill levels (Figure 2). Buffers can be pipetted or carefully poured to the appropriate volume.

    Note: Endotoxin Removal Wash can greatly reduce contaminants such as endotoxin, protein, RNA and endonucleases. For ease of use, the PureYield™ Midiprep Column is labeled with 5, 10 and 20ml fill levels (Figure 2). Buffers can be pipetted or carefully poured to the appropriate volume.

13. Add 20ml Column Wash Solution to the column and allow the vacuum to draw the solution through.

14. Dry the membrane by applying a vacuum for 30 seconds. After drying, the tops of the DNA binding membranes should appear dry and there should be no detectable ethanol odor.

    If the DNA binding membrane tops appear wet or there is a detectable ethanol odor, **repeat** the vacuum dry step for an additional 30 seconds.

15. Remove the PureYield™ 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. Place the column into a new 50ml disposable plastic tube (e.g., Corning or Falcon™).

5.C. DNA Purification by Quick Combination Method (continued)

Elute

16. To elute the DNA, place the binding column in a new 50ml disposable plastic tube (e.g., Corning or Falcon™), and add 600µl Nuclease-Free Water to the DNA binding membrane in the PureYield™ Binding Column.

    Note: Elution volume will affect both yield and concentration. The recommended elution volume, 600µl, was chosen to optimize the tradeoff between yield and concentration. If yield is more important than concentration, increase the elution volume. If concentration is more important than yield, decrease the elution volume. Elution volume should not be less than 400µl or plasmid recovery will be poor. Additional plasmid may be obtained with a second elution, although purity may be affected. Total volume recovered from both elutions should not exceed 1.0ml. Volumes eluted from the columns are typically less (25–50%) than the volume added due to rehydration of the membrane.

17. Centrifuge the PureYield™ Binding Column at 1,500–2,000 × *g* for 5 minutes.

18. Collect the filtrate from the 50ml tube and transfer to a 1.5ml tube if desired.

    Note: If a higher concentration is desired for subsequent applications, perform an ethanol precipitation (see Section 6 for protocol).

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

Plasmid DNA can be purified from overnight cultures of *E. coli* with the PureYield™ Plasmid Midiprep System. The yield of plasmid 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 2.0–4.0 for high-copy-number plasmids ensures that bacteria have reached the proper growth density for harvesting and plasmid DNA isolation.

5.E. Choosing a Bacterial Strain

Endonuclease I is a 12kDa periplasmic protein that degrades double-stranded DNA. This protein is encoded by the gene *end*A. The *E. coli* genotype *end*A1 refers to a mutation in the wildtype *end*A gene, which produces an inactive form of the nuclease. *E. coli* strains with this mutation are referred to as *EndA*–. Table 6 contains a list of *EndA*– and *EndA*+ *E. coli* strains.

Table 6. *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
KRX	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 Midiprep System, high-quality DNA is easily obtained from both *EndA*+ and *EndA*– bacterial strains.

5.F. Composition of Buffers and Solutions

Cell Resuspension Solution (CRA)

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

Cell Lysis Solution (CLA)

0.2M NaOH
1% SDS

Neutralization Solution (NSB)

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 purified	Ethanol was not added to Column Wash Solution. Prepare the Column Wash Solution as instructed before beginning the procedure.
	Clearing membrane was clogged. Clogging may be due to: • Too much cell mass. Use less biomass or non-rich medium. Lysate clearing by centrifugation (e.g., standard protocol, Section 4) may be more effective than vacuum clearing in cases of high cell mass. • Cell debris not allowed to float to top. Note incubation times listed in protocols.

Symptoms	Causes and Comments
Lysate is cloudy after clearing step when using alternative protocols	SDS precipitation may have occurred. When working with culture volumes less than 50ml, or with sparsely populated cultures ($O.D._{600}$ <2.0 O.D./ml), SDS from the Cell Lysis Solution can precipitate, making filter clearing difficult. When using cultures containing few cells or low volumes, decrease the Cell Resuspension, Cell Lysis and Neutralization Solution volumes.
Lysate has not moved through the Clearing Column	Clearing Column is clogged. If no preclearing centrifugation was performed, the vacuum may not be strong enough to pull the lysate through. Place the Clearing Column with the lysate in a 50ml tube and centrifuge at 1,500–2,000 × *g* for 5 minutes. Add the resulting lysate directly to the PureYield™ Binding Column.
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.
	Wrong reagents used. Make certain that Column Wash Solution 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 Midiprep System.
	Plasmid DNA yield not accurately quantitated. Quantitation by absorbance at A_{260} may over-estimate yield due to absorbance by a variety of factors such as RNA and protein. Use agarose gel/ethidium bromide quantitation.
	For plasmids greater than 10kb, yield may be increased by heating the water to 65°C at the elution step. Add Nuclease-Free Water to Binding Column and let sit for 1 minute. Elute as normal.

6. Troubleshooting (continued)

Symptoms	Causes and Comments
Low plasmid DNA yields (continued)	Some bacterial cells are more resistant to lysis and may require incubation for up to 5 minutes for efficient lysis. The lysate may not appear completely clear, but do not extend the lysis time beyond 5 minutes, as this may result in the formation of nicked or single-stranded DNA in the preparation.
Denaturation of plasmid DNA	Overincubation during lysis step. 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.
Ethanol carryover	Ethanol carryover is detected in the final product using the centrifugation method. After the wash step in the centrifugation protocol, transfer the binding column to a new 50ml tube and repeat centrifugation for 5 minutes to remove residual ethanol.
	Ethanol Wash Solution 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.
Lower-than-expected concentration of plasmid DNA	**Perform an ethanol precipitation.** Add 1/10 volume 3M sodium acetate (pH 5.2), 2.5 volumes 95% ethanol. Place on ice for 15 minutes. Pellet the DNA by centrifugation at 14,000 × *g* for 10 minutes in a microcentrifuge. Wash pellet with 70% ethanol and centrifuge at 14,000 × *g* for 10 minutes. Resuspend DNA pellet in desired volume of nuclease-free water.

7. Related Products

Product	Size	Cat.#
PureYield™ Plasmid Maxiprep System	10 preps	A2392
	25 preps	A2393
Cell Resuspension Solution (CRA)	315ml	A7115
Cell Lysis Solution (CLA)	315ml	A7125
Eluator™ Vacuum Elution Device	each	A1071
Neutralization Solution (NSB)	500ml	A1485
Vac-Man® Laboratory Vacuum Manifold, 20-sample capacity	each	A7231
Vac-Man® Jr. Laboratory Vacuum Manifold, 2-sample capacity	each	A7660
TNT® T7 Quick Coupled Transcription/Translation System*	40 reactions	L1170
TransFast™ Transfection Reagent	1.2mg	E2431
GoTaq® DNA Polymerase*	100u	M3001
	500u	M3005
GoTaq® Colorless Master Mix*	100 reactions	M7132
	1,000 reactions	M7133
GoTaq® Green Master Mix*	100 reactions	M7122
	1,000 reactions	M7123
GoTaq® Hot Start Colorless Master Mix*	100u	M5132
	1,000u	M5133
GoTaq® Hot Start Green Master Mix*	100u	M5122
	1,000u	M5123

*For Laboratory Use.
Catalog numbers may be different in Europe.

[a]U.S. Pat. No. 6,194,562, Australian Pat. No. 740145 and Canadian Pat. No. 2,329,067 have been issued to Promega Corporation for endotoxin reduction in nucleic acid purification. Other patents are pending.