Technical Bulletin

PureYield™ Plasmid Miniprep System

INSTRUCTIONS FOR USE OF PRODUCTS A1220, A1221, A1222 AND A1223.

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PureYield™ Plasmid Miniprep System

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1. Description

The PureYield™ Plasmid Miniprep System(a) isolates high-quality plasmid DNA for use in eukaryotic transfection and in vitro expression experiments. The system provides a rapid method to purify plasmid DNA using a silica-membrane column. Plasmid DNA can be purified in less than 10 minutes, depending on the number of samples processed, greatly reducing the time spent compared to silica resin or other membrane column methods.

The PureYield™ Plasmid Miniprep System incorporates a unique Endotoxin Removal Wash to remove 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 or extensive centrifugation, providing rapid purification of highly concentrated plasmid DNA.

1. Description (continued)

The PureYield™ Plasmid Miniprep System is designed to purify 1.5–7.5µg of plasmid DNA (Figure 1) with an A_{260}/A_{280} ≥1.8 from a 0.6ml overnight culture of bacteria transformed with a high-copy-number plasmid, with a total biomass ($O.D._{600}$ of culture × volume of culture in µl) of 1.3–8. If higher yields are desired, 1.5–3.0ml of culture can be processed. When working with low-copy-number plasmids, 3ml culture volumes are recommended.

This system can be used to isolate any plasmid from *E. coli* hosts but works most efficiently when the plasmid is up to 20,000bp in size. For best results, use plasmids that are 10,000bp or less. Purified plasmid can be used without further manipulation for automated fluorescent DNA sequencing as well as for other standard molecular biology techniques. When used for in vitro transcription reactions, the isolated plasmid DNA should be supplemented with a ribonuclease inhibitor such as Recombinant RNasin® Ribonuclease Inhibitor (Cat.# N2511). The protocol presented in this Technical Bulletin describes isolation of plasmid DNA from *E. coli*. Plasmid yield will vary, depending on a number of factors, including culture volume, plasmid copy number, type of culture medium and bacterial strain used. Section 5 provides information about how choice of bacterial strain can affect plasmid DNA yield.

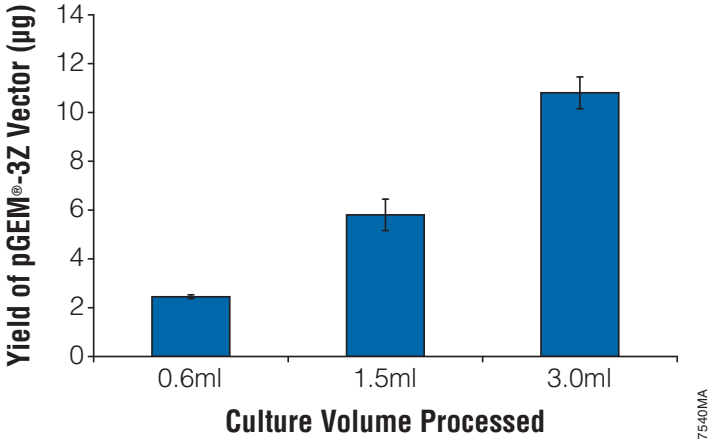

Figure 1. Plasmid DNA yield. pGEM®-3Z DNA was isolated from the indicated culture volumes using the PureYield™ Plasmid Miniprep System.

2. Product Components and Storage Conditions

Product	Size	Cat.#
PureYield™ Plasmid Miniprep System	10 preps	A1220

Includes:

- 1ml Cell Lysis Buffer (CLC) (Blue)
- 4ml Neutralization Solution (NSC)
- 2.5ml Endotoxin Removal Wash (ERB)
- 1ml Column Wash Solution (CWC)
- 0.5ml Elution Buffer (EBB)
- 10 PureYield™ Minicolumns
- 10 PureYield™ Collection Tubes

Product	Size	Cat.#
PureYield™ Plasmid Miniprep System	50 preps	A1221

Includes:

- 5.5ml Cell Lysis Buffer (CLC) (Blue)
- 18ml Neutralization Solution (NSC)
- 10.5ml Endotoxin Removal Wash (ERB)
- 4.6ml Column Wash Solution (CWC)
- 3ml Elution Buffer (EBB)
- 50 PureYield™ Minicolumns
- 50 PureYield™ Collection Tubes

Product	Size	Cat.#
PureYield™ Plasmid Miniprep System	100 preps	A1223

Includes:

- 11ml Cell Lysis Buffer (CLC) (Blue)
- 36ml Neutralization Solution (NSC)
- 20.5ml Endotoxin Removal Wash (ERB)
- 9ml Column Wash Solution (CWC)
- 10ml Elution Buffer (EBB)
- 2 × 50 PureYield™ Minicolumns
- 2 × 50 PureYield™ Collection Tubes

Product	Size	Cat.#
PureYield™ Plasmid Miniprep System	250 preps	A1222

Includes:

- 27ml Cell Lysis Buffer (CLC) (Blue)
- 90ml Neutralization Solution (NSC)
- 2 × 27ml Endotoxin Removal Wash (ERB)
- 25ml Column Wash Solution (CWC)
- 10ml Elution Buffer (EBB)
- 250 PureYield™ Minicolumns
- 250 PureYield™ Collection Tubes

2. Product Components and Storage Conditions (continued)

Storage Conditions: Store the Neutralization Solution (NSC) at 4–8°C, so that it is chilled and ready for use. Store all other components at room temperature.

Components of the Cell Lysis Buffer (CLC) may have precipitated during shipping. To completely resuspend the buffer, incubate the bottle at 30–37ºC for 30 minutes, and mix by inversion. Do not heat in the microwave.

Cell Lysis Buffer (CLC) contains NaOH, and Endotoxon Removal Wash (ERB) contains chaotropic reagents. Please use proper safety precautions with these reagents.

Note: Throughout the remainder of this document, the supplied Cell Lysis Buffer (CLC), Neutralization Solution (NSC), Endotoxin Removal Wash (ERB), Column Wash Solution (CWC) and Elution Buffer (EBB) are referred to as Cell Lysis Buffer, Neutralization Solution, Endotoxin Removal Wash, Column Wash Solution and Elution Buffer, respectively.

3. Before You Begin

Before you begin dilute the Column Wash Solution (provided) with 95% ethanol as described in Table 1.

Mark the bottle label to record that these additions have been made.

Table 1. Volume of 95% Ethanol to Add to the Column Wash Solution.

Cat.#	Number of Preps	Volume of 95% Ethanol	Total Volume
A1220	10	4ml	5ml
A1221	50	24ml	28.6ml
A1223	100	36ml	45ml
A1222	250	100ml	125ml

4. PureYield™ Plasmid Miniprep System Protocol

4.A. Centrifugation Protocol

Check the Cell Lysis Buffer to be sure that components have not precipitated during shipping. If precipitation has occurred, resuspend the buffer by incubating the bottle at 30–37ºC for 30 minutes and mixing by inversion.

We recommend the use of LB medium. If you are using other growth media, pellet the cells and resuspend the cell pellet in TE buffer or water prior to lysis.

To obtain higher DNA yields and greater purity for more demanding applications such as transfection or coupled transcription/translation, use the protocol in Section 4.C to process a minimum starting culture volume of 1.5ml. For the highest purity plasmid DNA, pelleting cells is recommended because contaminants accumulate in the cell medium during culture and may result in lower A_{260}/A_{230} ratios.

The following procedure is performed at room temperature.

1. Transfer 600µl of bacterial culture grown in LB medium to a 1.5ml microcentrifuge tube.

   Note: If you wish to process larger volumes of bacterial culture (up to 3.0ml), use the protocol provided in Section 4.C.

2. Add 100µl of Cell Lysis Buffer, and mix by inverting the tube 6 times.

   The solution should change from opaque to clear blue, indicating complete lysis.

   Note: Proceed to Step 3 within 2 minutes. Excessive lysis can result in denatured plasmid DNA. If processing a large number of samples, process samples in groups of ten or less. Continue with the next set of ten samples after the first set has been neutralized and mixed thoroughly.

3. Add 350µl of cold (4–8°C) Neutralization Solution, and mix thoroughly by inverting the tube.

   The sample will turn yellow when neutralization is complete, and a yellow precipitate will form. Invert the sample an additional 3 times to ensure complete neutralization.

4. Centrifuge at maximum speed in a microcentrifuge for 3 minutes.

5. Transfer the supernatant (~900µl) to a PureYield™ Minicolumn.

   Do not disturb the cell debris pellet. For maximum yield, transfer the supernatant with a pipette.

6. Place the minicolumn into a PureYield™ Collection Tube, and centrifuge at maximum speed in a microcentrifuge for 15 seconds.

7. Discard the flowthrough, and place the minicolumn into the same PureYield™ Collection Tube.

8. Add 200µl of Endotoxin Removal Wash to the minicolumn. Centrifuge at maximum speed in a microcentrifuge for 15 seconds. It is not necessary to empty the PureYield™ Collection Tube.

9. Add 400µl of Column Wash Solution to the minicolumn. Centrifuge at maximum speed in a microcentrifuge for 30 seconds.

4.A. Centrifugation Protocol (continued)

10. Transfer the minicolumn to a clean 1.5ml microcentrifuge tube, then add 30µl of Elution Buffer directly to the minicolumn matrix. Let stand for 1 minute at room temperature.

    Notes:

    1. Nuclease-free water at neutral pH can also be used to elute DNA.
    2. For large plasmids (>10kb), warm the Elution Buffer to 50ºC prior to elution, and increase elution volume to 50µl. Also incubate the column at room temperature (22–25°C) for 5–10 minutes before proceeding to Step 11.

11. Centrifuge at maximum speed in a microcentrifuge for 15 seconds to elute the plasmid DNA. Cap the microcentrifuge tube, and store eluted plasmid DNA at –20°C.

4.B. Vacuum Protocol

Check the Cell Lysis Buffer to be sure that components have not precipitated during shipping. If precipitation has occurred, resuspend the buffer components by incubating the bottle at 30–37ºC for 30 minutes and mixing by inversion.

We recommend the use of LB medium. If you are using other growth media, pellet the cells and resuspend the cell pellet in TE buffer or water prior to lysis.

The following procedure is performed at room temperature.

1. Transfer 1.5ml of bacterial culture to a 1.5ml microcentrifuge tube

   Note: If you wish to process larger volumes of bacterial culture (up to 3.0ml), use the protocol provided in Section 4.C.

2. Centrifuge at maximum speed in a microcentrifuge for 1 minute.

3. Remove and discard medium.

4. Resuspend the cell pellet in 600µl of TE buffer or water.

5. Add 100µl of Cell Lysis Buffer, and mix by inverting the tube 6 times.

   The solution should change from opaque to clear blue, indicating complete lysis.

   Note: Proceed to Step 6 within 2 minutes. Excessive lysis can result in denatured plasmid DNA. If processing a large number of samples, process samples in groups of ten or less. Continue with the next set of ten samples after the first set has been neutralized and mixed thoroughly.

6. Add 350µl of cold (4–8°C) Neutralization Solution, and mix thoroughly by inverting the tube. The sample will turn yellow when the neutralization is complete, and a yellow precipitate will form. Invert the sample an additional 3 times to ensure complete neutralization.

7. Centrifuge at maximum speed in a microcentrifuge for 3 minutes. Place a PureYield™ Minicolumn on a Luer-Lok® adapter of a VacMan® or VacMan® Jr. Laboratory Vacuum Manifold.
8. Transfer the supernatant (~900µl) into a PureYield™ Minicolumn.

   Do not disturb the cell debris pellet.
9. Apply vacuum to pull the lysate through the column.
10. Add 200µl of Endotoxin Removal Wash to the minicolumn. Allow the vacuum to pull the solution through the column.
11. Add 400µl of Column Wash Solution to the minicolumn. Allow the vacuum to pull the solution through the column. Release the vacuum, and remove the PureYield™ Minicolumn
12. Place the column in a PureYield™ Collection Tube, and centrifuge at maximum speed in a microcentrifuge for 1 minute.
13. Transfer the minicolumn to a clean 1.5 ml microcentrifuge tube, then add 30µl of Elution Buffer directly to the minicolumn matrix. Let stand for 1 minute at room temperature.

    Note: Nuclease-free water at neutral pH can also be used to elute the DNA.
14. Centrifuge at maximum speed in a microcentrifuge for 15 seconds to elute the plasmid DNA. Cap the microcentrifuge tube, and store eluted plasmid DNA at –20°C.

4.C. Alternative Protocol for Larger Culture Volumes

This alternative protocol can be used with the PureYield™ Plasmid Miniprep System to harvest and process up to 3ml of bacterial culture. We recommend using 3.0ml of bacterial culture when using low-copy-number plasmids.

1. Centrifuge 1.5 ml of bacterial culture for 30 seconds at maximum speed in a microcentrifuge.
2. Discard the supernatant.
3. To process a total of 3.0ml of culture, add an additional 1.5 ml of bacterial culture to the same tube. Repeat Steps 1 and 2.
4. Add 600µl of TE buffer or water to the cell pellet, and resuspend completely.
5. Proceed to Step 2 of the PureYield™ Plasmid Miniprep System protocol (Section 4.A).

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

Plasmid DNA can be purified from overnight cultures of *E. coli* with the PureYield™ Plasmid Miniprep System. Plasmid yield will vary depending on a number of factors, including plasmid copy number, cell density of bacterial culture, type of culture medium and bacterial strain used.

Plasmid copy number is an important factor affecting 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 overnight (12–16 hours) at 37°C. An A_{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.

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 A_{260}/A_{230} ratios	Overgrowth of cultures. Cell byproducts accumulated in the medium, resulting in lower A_{260}/A_{230} ratios; use cultures incubated for less than 20 hours.
	Some medium components may cause lower A_{260}/A_{230} ratios due to their affinity for the PureYield™ Minicolumn membrane. We recommend LB medium for direct culture lysis. Other media should be removed by centrifugation and cell pellets resuspended in water or TE buffer prior to lysis.
	Use the protocol in Section 4.C to pellet the cells prior to plasmid purification. Plasmid DNA with highest purity is obtained when cultures are pelleted and resuspended in water or TE buffer prior to lysis.

Symptoms	Causes and Comments
Poor cell lysis	Too many bacterial cells. Use cultures grown to an A_{600} of 2–4. All media should contain antibiotic. Use only recommended culture volumes for low- and high-copy-number plasmids (up to 3ml culture volume).
	Poor resuspension of bacterial cell pellet. Thoroughly resuspend cell pellets prior to cell lysis. Vortex or pipet pellet with Cell Resuspension Solution. No cell clumps should be visible after resuspension.
No plasmid DNA purified	Inaccurate quantitation of plasmid DNA yield. Quantitate plasmid DNA yield by agarose gel electrophoresis and ethidium-bromide staining.
	Low-copy-number plasmid was used, but only 0.6ml of culture was processed. Use 3.0ml of bacterial culture with low-copy-number plasmids.
	Poor aeration of culture. The optimal ratio of culture volume to air volume is 1:4 or less (20% culture, 80% air). For best aeration, use baffled culture flasks or a vented or gas-permeable seal on the culture vessel, and incubate with vigorous shaking.
	Incorrect culture medium. LB medium is recommended for the direct lysis method described in Section 4.A. Other culture media are not recommended for direct lysis but can be used with the alternative protocol (Section 4.C).
Low plasmid DNA yields	Use a larger culture volume. The standard centrifugation protocol uses 600µl of culture. Greater yields can be obtained by processing 1.5–3.0ml of bacterial culture (Sections 4.B and 4.C).
	Transfer of cleared lysate to column was nonoptimal. While the cleared lysate can be transferred to the PureYield™ Minicolumn by pouring, maximum yields are obtained when the supernatant is transferred completely by pipetting.
	Overgrowth of bacterial culture by nontransformed cells. Make certain that antibiotics are used in all media, both liquid and solid.

6. Troubleshooting (continued)

Symptoms	Causes and Comments
Low plasmid DNA yields (continued)	Bacterial culture was too old. Inoculate antibiotic-containing medium with a freshly isolated bacterial colony from an overnight plate. Incubate at 37°C for 12–16 hours.
	Low-copy-number plasmid was used. When using low-copy-number plasmids, use a larger culture volume (Section 4.C). Even with larger culture volumes, DNA yields will be lower than those with high-copy-number plasmids
	Plasmid DNA yield was not accurately quantitated. Use agarose gel electrophoresis and ethidium-bromide staining. Do not rely on spectrophotometry for quantitation.
	For large plasmids (>10kb), warm the Elution Buffer to 50ºC prior to elution, and increase the elution volume to 50µl. Also incubate the column at room temperature (22–25°C) for 5–10 minutes before centrifugation
Nicked plasmid DNA	Excessive incubation during alkaline lysis. Do not exceed a 2-minute incubation in Cell Lysis Buffer.
Poor results with automated fluorescent sequencing	Too little DNA was added to the sequencing reaction. Inoculate fresh LB medium with a newly isolated *E. coli* colony. Purify plasmid DNA and quantitate by agarose gel electrophoresis and ethidium-bromide staining.
	TE buffer was used for DNA elution. Repurify plasmid DNA, and elute in nuclease-free water.
	Plasmid concentration was not accurately quantitated. Perform agarose gel electrophoresis and ethidium-bromide staining to accurately quantitate plasmid DNA.
No restriction digestion	Increase restriction enzyme concentration or digestion time. Incubate at the recommended temperature and in the optimal reaction buffer for the restriction enzyme used.
	Ethanol precipitate the plasmid DNA to remove any salts that may have carried over.
	Insufficient centrifugation. Be sure that all centrifugation steps are performed at maximum speed in a microcentrifuge.

Symptoms	Causes and Comments
Genomic DNA contamination	Vortexing or overmixing during cell lysis resulted in genomic DNA contamination. Do not vortex samples after adding the Cell Lysis Solution to prevent shearing of genomic DNA.
	The wrong reagents were used. Use only PureYield™ Plasmid Miniprep System components. PureYield™ Plasmid Miniprep and Wizard® *Plus* SV System components are not interchangeable.

7. Composition of Buffers and Solutions

Elution Buffer

10mM Tris-HCl (pH 8.5)
0.1mM EDTA

8. Related Products

Product	Size	Cat.#
PureYield™ Plasmid Midiprep System	25 preps	A2492
	100 preps	A2495
PureYield™ Plasmid Maxiprep System	10 preps	A2392
	25 preps	A2393

[a]U.S. Pat. No. 6,194,562, Australian Pat. No. 740145, Canadian Pat. No. 2,329,067 and other patents pending.

[b]Lysis/neutralization indicator licensed under pending patents assigned to Zymo Research Corporation.