

PureYield™ Plasmid Midiprep System

The PureYield Plasmid Midiprep System^(a) provides high-speed purification of plasmid DNA using a newly developed silica-membrane-based column. This unique design allows purification of 100–200µg of plasmid DNA from 50ml of bacterial culture in 30 minutes. Elution is performed in a small volume of water, providing concentrated DNA for your chosen application, saving time and the extra work of performing an isopropanol precipitation after eluting DNA. An Endotoxin Removal Wash is used to reduce endotoxin, protein and RNA contamination, improving results for eukaryotic cell transfection, in vitro transcription/translation reactions and fluorescent DNA sequencing.

Procedure: After alkaline lysis of transformed bacteria, lysate clearing is accomplished using the PureYield Clearing Column in a spin or vacuum format. When using the vacuum protocol, the nested set-up of the PureYield Clearing Column and the PureYield Binding Column allows rapid clearing and DNA binding to occur in one step. Once plasmid DNA is bound to the PureYield Binding Column, a single wash with the Endotoxin Removal Wash is performed followed by a single wash with the Column Wash. These washes are rapid and typically take less than 5 minutes to complete. Highly pure concentrated DNA is eluted with water.

Benefits:

- **Have confidence in your results:** High purity and concentration of plasmid DNA gives proven performance in transfection, in vitro expression and other molecular biology applications.
- **Improve your productivity:** Rapid protocol purifies plasmid DNA in 30 minutes.
- **Ease of use:** Simple protocol eliminates tedious high-speed centrifugation, gravity-drip columns, and post-elution alcohol precipitation.
- **Flexibility:** PureYield membrane-column allows purification of large amounts of plasmid DNA exceeding the capabilities of other midiprep systems.

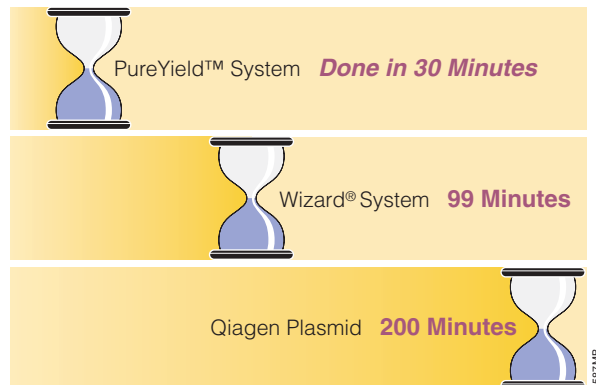


Figure 1. Comparison of time required per midiprep using different systems. Each system protocol was performed according to the manufacturer's instructions using 50ml of an overnight culture of JM109 bacteria transformed with a high copy plasmid (pGEM®-3 plasmid). Total time to perform the midiprep is noted.

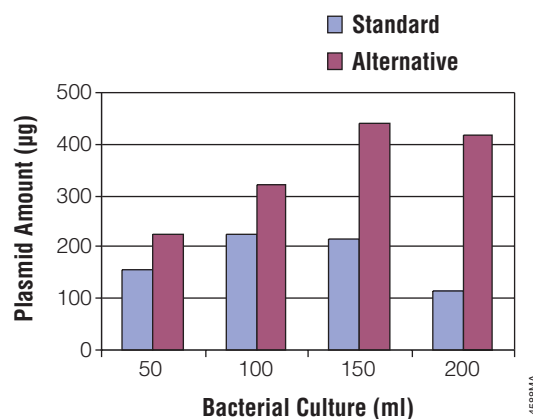


Figure 2. Comparison of plasmid yield with increasing total biomass. Increasing amounts of JM109 containing pGEM®-3 plasmid were grown and processed using the PureYield Plasmid Midiprep System. Lysate was prepared using either the standard vacuum protocol or alternative lysate preparation protocol. The use of the high speed spin to facilitate clearing allows the user to efficiently process large quantities of culture, providing maxiprep yield from a midiprep.

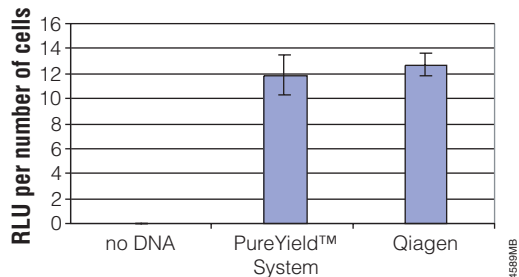
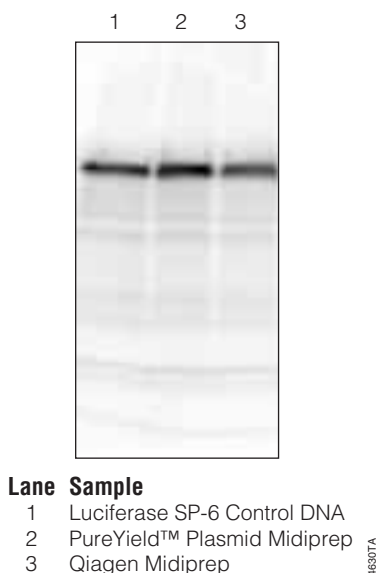


Figure 3. Comparison of transfection efficiency of purified plasmid DNA prepared using different midprep systems. HeLa cells were transfected with pSV-βgal (Cat.# PAE1081) in a 96-well plate format using 0.1μg plasmid/well. Twenty-four hours after transfection, β-galactosidase activity (using the Beta-Glo® Assay System Cat.# PAE4720) and cell density (using the CellTiter-Glo® Luminescent Cell Viability Assay Cat.# PAG7570) were monitored with a Fluor-star luminometer. The experiments were performed in triplicate.



Lane Sample
 1 Luciferase SP-6 Control DNA
 2 PureYield™ Plasmid Midprep
 3 Qiagen Midprep

Figure 4. Comparison of in vitro transcription/translation results of purified plasmid DNA prepared using different midprep systems. Midpreps of the Luciferase SP6 Control DNA (Cat.# PAL4741) were performed as directed by the manufacturer. Transcription/translation reactions were performed using the TNT® SP6 Quick Coupled Transcription/Translation System (Cat.# PAL2080) according to supplied Technical Manual. One microliter of each in TNT® reaction was analyzed by SDS-PAGE on a 4-20% NOVEX® gel. The separated proteins were transferred to a PVDF membrane (BioRad, Sequi-Blot) and then exposed for 2 hours to a PhosphorImager cassette. A Storm® PhosphorImager was used to analyze the cassette.



Figure 5. Proper assembly of the PureYield Clearing Column (blue) and the PureYield DNA Binding Column (white) for use with the vacuum protocol of the PureYield Plasmid Midprep System.

Ordering Information

| Product | Size | Cat. # |
|--|-----------|---------|
| PureYield™ Plasmid Midprep System ^(a) | 25 preps | PAA2492 |
| | 100 preps | PAA2495 |

Related Products

| Product | Size | Cat. # |
|--|-----------------|---------|
| Vac-Man® Laboratory Vacuum Manifold, 20-sample capacity | each | PAA7231 |
| Vac-Man® Jr. Laboratory Vacuum Manifold, 2-sample capacity | each | PAA7660 |
| TnT® T7 Quick Coupled Transcription/Translation System ^{(b,c,d,e,f)*} | 40 reactions | PAL1170 |
| TransFast™ Transfection Reagent ^(g) | 1.2mg | PAE2431 |
| GoTaq® DNA Polymerase ^{(h)*} | 100u | PAM3001 |
| | 500u | PAM3005 |
| PCR Master Mix ^{(i)*} | 100 reactions | PAM7502 |
| | 1,000 reactions | PAM7505 |

*For Laboratory Use.

Additional Information

| Literature | Part # |
|--|--------|
| PureYield™ Plasmid Midprep System Technical Manual www.promega.com/tbs/tm253/tm253.html | TM253 |

Products may be covered by pending or issued patents. Please visit our website for more information.

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NOVEX is a registered trademark of Novel Experimental Technologies.

Storm is a registered trademark of Amersham Biosciences Ltd.

^(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.

^(b) U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289 and 5,814,471, Australian Pat. No. 649289, European Pat. No. 0 553 234 and Japanese Pat. No. 3171595 have been issued to Promega Corporation for a beetle luciferase assay method, which affords greater light output with improved kinetics as compared to the conventional assay. Other patents are pending.

^(c) U.S. Pat. Nos. 5,324,637 and 5,492,817, European Pat. No. 0 566 714 B1, Australian Pat. No. 660329 and Japanese Pat. No. 2904583 have been issued to Promega Corporation for coupled transcription/translation systems that use RNA polymerases and eukaryotic lysates.

^(d) U.S. Pat. No. 5,552,302, European Pat. No. 0 422 217, Australian Pat. No. 646803 and Japanese Pat. Nos. 3009458 and 3366596 have been issued to Promega Corporation for the methods and compositions for production of human recombinant placental ribonuclease inhibitor.

^(e) The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.

^(f) U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, Australian Pat. Nos. 616881 and 641261 and other pending and issued patents, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.

^(g) The cationic lipid component of the TransFast™ Transfection Reagent is covered by U.S. Pat. Nos. 5,824,812, 5,869,715 and 5,925,623, Australian Pat. No. 713093 and pending foreign patents.

^(h) Certain applications of this product are covered by patents issued and applicable in certain countries. Because purchase of this product does not include a license to perform any patented application, users of this product may be required to obtain a patent license depending upon the particular application and country in which the product is used.

⁽ⁱ⁾ The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.



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