Technical Bulletin

Wizard® *Plus* Megapreps DNA Purification System

INSTRUCTIONS FOR USE OF PRODUCTS A7300, A7361 AND A7421.

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Wizard® *Plus* Megapreps DNA Purification System

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1. Description

Large-scale plasmid preparations, such as cesium chloride purification, can be both laborious and time-consuming, often requiring an overnight centrifugation (1). The Wizard® *Plus* Megapreps DNA Purification System[a] is simple and rapid, requiring only a centrifuge, a vacuum source and a vacuum manifold such as the Vac-Man® (20-sample capacity, Cat.# A7231) or Vac-Man® Jr. (2-sample capacity, Cat.# A7660) Laboratory Vacuum Manifold. The system yields greater than one milligram of high-copy-number plasmid DNA (200–20,000bp) from a 1,000ml culture in less than three hours.

The Wizard® *Plus* Megapreps System does not require organic extractions or cesium chloride gradients. The purified DNA is eluted in Nuclease-Free Water (Cat.# P1193) or TE buffer. The purified plasmid can be used directly for DNA sequencing and restriction digestion without further manipulation and also can be used for in vitro transcription reactions supplemented with a ribonuclease inhibitor such as Recombinant RNasin® Ribonuclease Inhibitor(Cat.# N2511).

Selected Citations Using the Wizard® *Plus* Megapreps DNA Purification System

- Matsumoto, Y., Jee, Y. and Sugisaki, M. (2000) Successful TCR-based immunotherapy for autoimmune myocarditis with DNA vaccines after rapid identification of pathogenic TCR. *J. Immunol.* **164**, 2248–54.

  The T-cell receptor of interest was amplified and subcloned in the pTARGET™ Mammalian Expression Vector. Large amounts of the vector were purified with the Wizard® *Plus* Megapreps DNA Purification System and used as a DNA vaccine in Lewis rats.

- Jones, L.C. and Scammell, J.G. (1998) The cAMP-response element mediates induction of secretogranin II by CHX and FSK in GH4C1 cells. *Am. J. Physiol.* **274**, E656–64.

  Authors used the Wizard® *Plus* Megapreps DNA Purification System to isolate DNA and transfect it into GH cells.

For additional peer-reviewed articles that cite use of the Wizard® *Plus* Megapreps DNA Purification System, visit: **www.promega.com/citations/**

2. Product Components

Product	Size	Cat.#
Wizard® *Plus* Megapreps DNA Purification System	5 preps	A7300

Each system contains sufficient reagents and columns for 5 isolations from 500–1,000ml of bacterial culture (using EndA– strains). Includes:

- 150ml Cell Resuspension Solution
- 150ml Cell Lysis Solution
- 300ml Neutralization Solution
- 100ml Wizard® Megapreps DNA Purification Resin
- 125ml Column Wash Solution
- 5 Maxi/Megacolumns with Reservoirs
- 5 5ml Syringes
- 5 0.2µm Syringe Filters

Product	Size	Cat.#
Wizard® Megapreps DNA Purification Resin[a]	1,000ml	A7361
Wizard® Maxi/Megapreps Filtering System	50 each	A7421

Storage and Stability: All Wizard® *Plus* Megapreps components are guaranteed for at least 6 months from the date of purchase when stored at room temperature. No refrigeration is required. **Protect the resin from exposure to direct sunlight.**

3. Production of a Cleared Lysate

Materials to Be Supplied by the User
(Solution compositions are provided in Section 7.)

- ethanol (95%)
- TE buffer
- isopropanol (at 22–25°C)
- Miracloth™ (Calbiochem Corp. Cat.# 475855), filter paper (Whatman® #1, GFA or GFC) or an autoclaved coffee filter

Before you begin, dilute the Column Wash Solution (provided) by adding 170ml of 95% ethanol for a final volume of 295ml.

1. Pellet 500–1,000ml of cells by centrifugation at 1,500 × *g* for 20 minutes at 22–25°C in a room temperature rotor. Pour off the supernatant and resuspend the pellet in 30ml of Cell Resuspension Solution. (To aid resuspension, manually disrupt the pellet with a 12-inch applicator stick or by gently pipetting until no clumps are visible. Complete resuspension is **critical** for optimal yields.)

2. Add 30ml of Cell Lysis Solution and mix gently but thoroughly, by stirring or inverting. Do not vortex. Cell lysis is complete when the solution becomes clear and viscous (up to 20 minutes).

3. Add 30ml of Neutralization Solution and immediately mix by gently inverting the centrifuge bottle several times.

   If using an EndA+ strain, add 60ml of Neutralization Solution, mix by inverting the centrifuge bottle 10–20 times and incubate the lysate at room temperature for 10 minutes. Proceed to Step 4.

4. Centrifuge at 14,000 × *g* for 15 minutes at 22–25°C in a **room temperature** rotor.

5. Transfer the cleared supernatant by filtering it through Miracloth™ (Calbiochem Corp., Cat.# 475855), filter paper (Whatman® #1, GFA or GFC) or an autoclaved coffee filter into a clean 100ml graduated cylinder. Measure the supernatant volume, then transfer to a centrifuge bottle.

6. Add 0.5 volume of **room temperature** isopropanol and mix by inversion.

7. Centrifuge at 14,000 × *g* for 15 minutes at 22–25°C in a **room temperature** rotor.

8. Discard the supernatant and resuspend the DNA pellet in 4ml of TE buffer. Thoroughly wash the walls of the centrifuge bottle with TE buffer to recover all of the DNA. At this point, the pellet may not be visible.

4. Plasmid DNA Purification

A vacuum source and the Vac-Man® or Vac-Man® Jr. Laboratory Vacuum Manifold are required for this procedure. To dry the resin (Step 5) a centrifuge with a swinging bucket rotor is required.

Materials to Be Supplied by the User
(Solution compositions are provided in Section 7.)

- vacuum pump or vacuum aspirator capable of achieving a vacuum of 15–18 inches of mercury (Hg)
- vacuum manifold, e.g., Vac-Man® (Cat.# A7231) or Vac-Man® Jr. (Cat.# A7660) Vacuum Manifold
- ethanol (80%)
- centrifuge capable of 1,300–14,000 × *g*
- swinging bucket rotor (e.g., Beckman JS-4.3 rotor, required for Step 5)
- Nuclease-Free Water (Cat.# P1193) **preheated to 65–70°C**
- 50ml screw cap tubes
- **optional:** 40% isopropanol/4.2M guanidine hydrochloride solution (required for EndA+ strains; use only Promega Cat.# H5381 or Amresco Cat.# 0118 guanidine HCl)

Comparison of Inches of Hg to Other Pressure Measurements.
15 Inches Hg
50.8kPa
381 Torr
0.501atm
7.37psi
38.1cm Hg
508mbar

1. Add 20ml of Wizard® Megapreps DNA Purification Resin to the DNA solution from Section 3, Step 8. Swirl to mix.

Thoroughly mix the Wizard® Megapreps DNA Purification Resin before removing an aliquot.

2. For each Megaprep, use one Megacolumn. Insert the Megacolumn tip into the vacuum manifold port.

3. Transfer the resin/DNA mix into the Megacolumn. Apply a vacuum of at least 15 inches of Hg to pull the resin/DNA mix into the Megacolumn.

If using an EndA+ strain, add 25ml of 40% isopropanol/4.2M guanidine hydrochloride solution (Section 7) to each column. Apply a vacuum continuously until 30 seconds after all of the solution has flowed through the columns. Please note that this solution will flow through the column more slowly than the standard Column Wash Solution. Proceed to Step 4.

4. Add 25ml of Column Wash Solution to the Megacolumn and apply a vacuum to draw the solution through the Megacolumn. Repeat this procedure once.

5. To rinse the resin, add 5ml of 80% ethanol to the Megacolumn and apply a vacuum to draw the ethanol through the Megacolumn. Allow the vacuum to draw for an additional 1 minute.

6. Insert the Megacolumn into a 50ml screw cap tube (provided by the user). Using a centrifuge with a swinging bucket rotor (e.g., Beckman JS-4.3 rotor), centrifuge the Megacolumn at 2,500rpm (1,300 × *g*) for 5 minutes.

   It is **essential** that a swinging bucket rotor be used for this step.

   Remove the Megacolumn and discard both the tube and the liquid. Place the Megacolumn back on the vacuum manifold.

7. Dry the resin to completion by applying a vacuum for 5 minutes. Remove the Megacolumn from the vacuum manifold. Place the Megacolumn in the provided Reservoir (50ml screw cap tube).

   For elution of large plasmids (≥10kb), the use of water preheated to 65–70°C may increase yields. For plasmids ≥20kb, use water preheated to 80°C.

8. Add 3.0ml of preheated (65–70°C) nuclease-free water to the Megacolumn and wait 1 minute. Elute the DNA by centrifuging the Megacolumn/Reservoir at 2,500rpm (1,300 × *g*) for 5 minutes in a centrifuge with a swinging bucket rotor or at 1,300 × *g* for 5 minutes in a centrifuge with a fixed-angle rotor (e.g., Beckman J2-21 centrifuge with JA-17 rotor).

9. A white pellet of resin fines may be present in the final eluate. Whether visible or not, it is important to separate the fines from the DNA. Remove the plunger from one of the 5ml Syringes and set it aside.

10. Attach the syringe barrel to the Luer-Lok® extension of one of the 0.2µm Syringe Filters and pipet the eluate into the Syringe Barrel.

11. Center the filter over a 15ml plastic tube. Carefully insert the plunger into the syringe barrel and gently push the liquid into the tube.

12. Transfer the eluate to 1.5ml centrifuge tubes. Centrifuge at 14,000 × *g* for 1 minute. This additional step will remove all resin fines that may be present in the final eluate.

13. Immediately transfer the supernatant to a new microcentrifuge tube. Follow these storage recommendations: DNA is stable in water without addition of buffer if stored at –20°C or below. DNA is stable at 4°C in TE buffer. To store the DNA in TE buffer, add 300µl of 10X TE buffer to the 3ml of eluted DNA.

5. Supplementary Information

Plasmid DNA can be purified from 500–1,000ml overnight cultures of *E. coli* with the Wizard® *Plus* Megapreps System. The yield of plasmid will vary depending on a number of factors, including the volume of bacterial culture, plasmid copy number, type of culture medium and the bacterial strain. The protocol presented in this technical bulletin is for the isolation of plasmid DNA from *E. coli*.

5.A. Factors Affecting Plasmid DNA Yield

Plasmid copy number is one of the most important factors affecting yield in a given system. Copy number is determined primarily by the region of DNA surrounding and including the origin of replication in the plasmid. This area, known as the replicon, controls replication of plasmid DNA by bacterial enzyme complexes. Some DNA sequences, when inserted into a particular vector, can lower the copy number of the plasmid. In addition, excessively large DNA inserts can reduce plasmid copy number. In many cases, the exact copy number of a particular construct will not be known. However, many of these plasmids will have been derived from a small number of commonly used parent constructs.

5.B. 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 in the *end*A gene are referred to as EndA negative (EndA–). Table 1 contains a list of EndA– and EndA+ *E. coli* strains. The wildtype is indicated as EndA+. Using the Wizard® *Plus* Megapreps System, high-quality DNA is easily obtained from both EndA+ and EndA– strains. Special precautions must be taken when working with EndA+ strains to ensure the isolation of high-quality DNA (2), including the use of several modified protocol steps, as indicated, and the use of a less rich growth medium (e.g., LB). The modified protocol will eliminate most problems associated with these strains. However, the level of endonuclease I produced is strain-dependent, and the modified protocol may not totally exclude endonuclease I from plasmid DNA prepared from very high endonuclease I-producing strains. Also note that the modified protocol requires the use of increased volumes of several of the supplied solutions, and as a result, you will be unable to perform as many isolations. In general, we recommend the use of EndA– strains whenever possible.

5.C. Special Considerations for Automated Fluorescent Sequencing

For the application of automated fluorescent sequencing, special consideration should be given to the selection of plasmid type and *E. coli* strain to optimize yield and plasmid quality. Optimal automated fluorescent sequencing results are routinely obtained by using high-copy-number plasmids and EndA– strains of *E. coli*.

Note: For fluorescent sequencing applications, elute and store the DNA in nuclease-free water.

Purified plasmid DNA must be within the proper concentration range for successful automated cycle sequencing (ideally 0.2µg/µl, not less than 0.1µg/µl). When working with plasmid DNA from low-copy-number plasmids, we strongly recommend that DNA concentrations be determined by agarose gel/ethidium bromide quantitation prior to any application. DNA

quantitation by spectrophotometric methods is prone to errors and requires a large amount of sample.

The Wizard® *Plus* Megapreps System routinely yields greater than 1,000µg of medium- or high-copy-number plasmid DNA when used with the pGEM® Vector and DH5α™ cells in 1,000ml culture.

Special Considerations for Sequencing Using BigDye® Chemistry

If the BigDye® terminator ready reaction mix (The Perkin-Elmer Corporation, Cat.# 43031-49) is diluted, it is essential to use an appropriate dilution buffer, such as 250mM Tris-HCl (pH 9.0), 10mM $MgCl_2$.

To ensure optimal sequencing results when using DNA prepared with the Wizard® *Plus* DNA Purification System in combination with ABI PRISM® BigDye® terminator cycle sequencing chemistries, it is essential that an ethanol precipitation step be performed after eluting the DNA from the column. The resulting DNA pellet should then be resuspended in nuclease-free water.

Table 1. List of EndA– and EndA+ Strains.

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	P2392
JM109	PR700 (all PR series strains are EndA+)
MM294	Q358
SK1590	RR1
SK1592	TB1
SK2267	TG1
SRB	Y1088 (all Y10 series strains are EndA+)
XL1-Blue	BMH71-18
XLO	ES1301

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 media 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 the Cell Resuspension Solution. No cell clumps should be visible after resuspension.
No plasmid DNA purified	Ethanol not added to the Column Wash Solution. Prepare the Column Wash Solution as instructed before beginning the procedure.
	EndA+ strain of bacteria used. DNA appears degraded or lost upon incubation with Mg^{2+} containing buffer (i.e., restriction enzyme buffer). Follow protocol modifications for EndA+ strains of bacteria.
	Inaccurate quantitation of plasmid DNA yield. Quantitate plasmid DNA yield by agarose gel/ethidium bromide electrophoresis.
DNA floats out of well during loading of agarose gel	Carryover of residual ethanol from Column Wash Solution. Follow directions for appropriate drying of resin by vacuum and centrifugation. If DNA has already been eluted, precipitate DNA and dry remaining ethanol from the DNA pellet prior to resuspension in nuclease-free water. Increase loading dye concentration to 2X.
Low plasmid DNA yields	Overgrowth of bacterial culture by nontransformed bacteria. Make certain that antibiotics were used in all media, both liquid and solid. Do not culture bacteria longer than 24 hours. Optimal culture length is 12–16 hours.
	Bacterial culture too old. Inoculate antibiotic containing media with freshly isolated bacterial colony from an overnight plate.

6. Troubleshooting (continued)

Symptoms	Causes and Comments
Low plasmid DNA yields (continued)	Low-copy-number plasmid used. See Section 5.A. Cultures should not exceed the maximum recommended volumes per isolation.
	Precipitate formed in resin. Warm resin in 37°C water bath for 15–20 minutes. Gently swirl to mix and allow to cool to 30°C prior to use.
	Presence of resin fines in eluted DNA. Follow directions for removal of resin fines from eluted DNA (i.e., filtration and centrifugation). If DNA aggregate has formed, heat in the presence of 1M NaCl to redissolve aggregate. Centrifuge to remove resin fines. Precipitate DNA with ethanol and wash with 70% ethanol to remove residual NaCl before use in downstream applications.
	Overdrying of resin on vacuum source. Follow directions for drying on vacuum source. Do not dry for times longer than suggested.
	Wrong reagents used. Make certain Column Wash Solution is diluted with ethanol before use. Note that Wizard® *Plus* and Wizard® *Plus* SV components are not interchangeable.
	Plasmid DNA yield not accurately quantitated. Use agarose gel/ethidium bromide quantitation.
Nicking of plasmid DNA	Overincubation during the alkaline lysis step. Total incubation of cell suspension with Cell Lysis Solution should not exceed 20 minutes.
No results or 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/ethidium bromide electrophoresis.
	TE buffer was used for DNA elution. Ethanol precipitate and resuspend pellet in nuclease-free water. (The EDTA in TE buffer can interfere with downstream applications by chelating Mg^{2+}.)
	Using ABI PRISM® BigDye® chemistry. Use of ABI PRISM® BigDye® sequencing chemistry necessitates ethanol precipitation of eluted DNA prior to sequencing reaction.

6. Troubleshooting (continued)

Symptoms	Causes and Comments
No results or poor results with automated fluorescent sequencing (continued)	Plasmid concentration not accurately quantitated. Ethidium bromide gel electrophoresis must be used to accurately quantitate plasmid DNA.
No restriction digestion	Concentration of restriction enzyme and length of digestion need to be optimized. Increase the amount of restriction enzyme and/or the length of incubation time. Digest at suggested temperature and in the optimal buffer for the restriction enzyme used.
	DNA degraded during restriction digestion due to use of EndA+ *E. coli* strain. Repurify DNA from fresh culture containing antibiotics. Follow instructions (Section 3 and 4) for EndA+ strains or use an EndA– strain of *E. coli*.
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.
DNA yields on gel look low compared to spectrophotometer readings	Traces of contaminants may be present in the eluted DNA, which inflate the spectrophotometer readings. Phenol:chloroform extract and precipitate DNA, then wash with 70% ethanol before repeating spectrophotometer readings. Alternatively, quantitate the DNA by agarose gel/ethidium bromide electrophoresis for more accurate quantitation.

7. Composition of Buffers and Solutions

Cell Resuspension Solution

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

Cell Lysis Solution

0.2M	NaOH
1%	SDS

Neutralization Solution

1.32M	potassium acetate (pH 4.8)

Column Wash Solution

80mM	potassium acetate
8.3mM	Tris-HCl (pH 7.5)
40µM	EDTA

Add 170ml of 95% ethanol (Section 3). Final ethanol concentration will be approximately 55%. (Component concentrations listed are for final solution with ethanol added.)

TE buffer (1X)

10mM	Tris-HCl (pH 7.5)
1mM	EDTA

40% isopropanol/4.2M guanidine HCl

66.9g	guanidine hydrochloride (use only Promega Cat.# H5381 or Amresco Cat. #0118)

Prepare a 7M solution by dissolving the guanidine hydrochloride in 50–60ml of sterile, distilled water. This reaction is very endothermic; warming the mixture to 37°C (do not exceed 37°C) will speed the process. Bring to a final volume of 100ml with sterile, distilled water.

Prepare the 40% isopropanol/4.2M guanidine HCl solution by combining 30ml of the 7M guanidine HCl solution with 20ml of isopropanol in a 50ml screw cap tube and mixing thoroughly. Store at room temperature.

8. Related Products

Product	Size	Cat.#
Wizard® *Plus* SV Minipreps DNA Purification System	50 preps	A1330
	250 preps	A1460
Wizard® *Plus* SV Minipreps DNA Purification System + Vacuum Adapters	50 preps	A1340
	250 preps	A1470
Wizard® *Plus* Minipreps DNA Purification System	50 preps	A7100
	100 preps	A7500
	250 preps	A7510
Wizard® *Plus* Midipreps DNA Purification System	25 preps	A7640
Wizard® *Plus* Maxipreps DNA Purification System	10 preps	A7270
Wizard® Minipreps DNA Purification Resin	250ml	A7141
Wizard® Midipreps DNA Purification Resin	1,000ml	A7701
Wizard® Maxipreps DNA Purification Resin	500ml	A7401
Wizard® SV 96 Plasmid DNA Purification System	1 × 96 preps	A2250
	5 × 96 preps	A2255
Cell Resuspension Solution	150ml	A7112
Cell Lysis Solution (Plasmid Purification)	150ml	A7122
Neutralization Solution	150ml	A7131
Column Wash Solution	125ml	A8102
Wizard® Minicolumns	250 each	A7211
Wizard® Midicolumns	100 each	A7651
Vac-Man® Laboratory Vacuum Manifold	20-sample capacity	A7231
Vac-Man® Jr. Laboratory Vacuum Manifold	2-sample capacity	A7660
Vac-Man® 96 Vacuum Manifold	96-sample capacity	A2291
One-Way Luer-Lok® Stopcocks	10 each	A7261

9. References

1. Ausubel, F.M. *et al.* (1989) *Current Protocols in Molecular Biology*, Vol. 2, John Wiley & Sons, New York.

2. Schoenfeld, T. *et al.* (1995) DNA purification: Effects of bacterial strains carrying the *end*A1 genotype on DNA quality isolated with Wizard® Plasmid Purification Systems. *Promega Notes* **53**, 12–20.

[a]U.S. Pat. Nos. 5,658,548 and 5,808,041, Australian Pat. No. 689815 and European Pat. No. 0 723 549 have been issued to Promega Corporation for nucleic acid purification on silica gel and glass mixtures. Other patents are pending.