Promega

Technical Bulletin

Wizard® *Plus* Minipreps DNA Purification System

INSTRUCTIONS FOR USE OF PRODUCTS A7100, A7500, A7510, A7141 AND A7211.

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Promega

Wizard® *Plus* Minipreps DNA Purification System

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I. Description

Small-scale purifications of plasmid DNA, better known as minipreps, are commonly used in molecular biology procedures. Over the years, many miniprep protocols have been used (1), but few have proven to be consistently reliable. The miniprep process can be both laborious and time-consuming, particularly when large numbers of minipreps are performed in parallel.

The Wizard® *Plus* Minipreps DNA Purification System[a], which eliminates many of the problems associated with standard miniprep procedures, provides a simple and reliable method for rapidly isolating plasmid DNA. This system can be used to isolate any plasmid but works most efficiently when the plasmid is <20,000bp. When using the standard protocol, the entire miniprep process can be completed in 15 minutes or less, with no organic extractions or ethanol

precipitations. Multiple minipreps may be easily processed at one time with the Vac-Man® (20-sample capacity, Cat.# A7231) or Vac-Man® Jr. (2-sample capacity, Cat.# A7660) Laboratory Vacuum Manifold. DNA is eluted from the Wizard® Minicolumn in nuclease-free water. The purified plasmid can be used directly for automated fluorescent 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* Minipreps DNA Purification System

- Magnusson, C., Libelius, R. and Tagerud, S. (2003) Nogo (Reticulon 4) expression in innervated and denervated mouse skeletal muscle. *Mol. Cell. Neurosci.* **22**, 298–307.

  cDNA fragments from the Nogo gene were amplified from genomic DNA and cloned into the pGEM®-T Vector. The Wizard® *Plus* Mini- and Midipreps kits were used to purify the plasmids from bacterial cells.

- Kemp, E.H. *et al.* (2002) The melanin-concentrating hormone receptor 1, a novel target of autoantibody responses in vitiligo. *J. Clin. Invest.* **109**, 923–30.

  T4 polynucleotide kinase was used to phosphorylate primers before ligating them into a phagemid vector to allow cloning of *Sfi* I-restricted DNA fragments. Phagemid was prepared from XL1-Blue cells using the Wizard® *Plus* Minipreps DNA Purification System.

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

II. Product Components

Product	Size	Cat.#
Wizard® *Plus* Minipreps DNA Purification System	50 preps	A7100

For Laboratory Use. Each system contains all the reagents required for either 50 standard minipreps from 1–3ml of culture, 30 standard minipreps from 3–5ml of culture or 25 standard minipreps from 5–10ml of culture (using EndA– strains). Includes:

- 10ml Cell Resuspension Solution
- 10ml Cell Lysis Solution
- 20ml Neutralization Solution
- 50ml Wizard® Minipreps DNA Purification Resin
- 50ml Column Wash Solution
- 50 Wizard® Minicolumns
- 50 Syringe Barrels (3ml)
- 1 Protocol

Product	Size	Cat.#
Wizard® *Plus* Minipreps DNA Purification System	100 preps	A7500
Wizard® *Plus* Minipreps DNA Purification System	250 preps	A7510

For Laboratory Use. These systems are identical to the Wizard® *Plus* Minipreps System for 50 standard minipreps, except that they contain 2 or 5 times, respectively, the amount of each component.

Product	Size	Cat.#
Wizard® Minipreps DNA Purification Resin*[(a)]	250ml	A7141
Wizard® Minicolumns	250 each	A7211

*For Laboratory Use.

Storage and Stability: All Wizard® *Plus* Minipreps 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.**

III. Production of a Cleared Lysate

When isolating high-copy-number plasmids, it is unnecessary to process more than 5ml of bacterial culture to obtain sufficient plasmid DNA for multiple molecular biology applications. If more than 5ml of culture is processed, the capacity of the resin will be exceeded and no increase in plasmid yield will be observed. However, when isolating low-copy-number plasmids, it is useful to process 5–10ml of bacterial culture.

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

Table 1. Amount of Ethanol to Add to Column Wash Solution.

Cat.#	# of Preps	Volume of 95% Ethanol	Total Volume
A7100	50	70ml	120ml
A7500	100	140ml	240ml
A7510	250	320ml	550ml

III.A. From 1–3ml of Bacterial Culture

1. Pellet 1–3ml of cells by centrifugation for 1–2 minutes at 10,000 × *g* in a microcentrifuge. Pour off the supernatant and blot the tube upside-down on a paper towel to remove excess media.
2. Completely resuspend the cell pellet in 200µl of Cell Resuspension Solution. Transfer the cells to a 1.5ml microcentrifuge tube if necessary.
3. Add 200µl of Cell Lysis Solution and mix by inverting the tube 4 times. The cell suspension should clear immediately.
4. Add 200µl of Neutralization Solution and mix by inverting the tube 4 times.

   Alternatively, if using an EndA+ strain, add 400µl of Neutralization Solution, mix by inverting the tube 4 times and incubate the lysate at room temperature for 10 minutes.

   Note: Some bacterial strains are more resistant to lysis and may require incubation for 3–5 minutes for efficient lysis.
5. Centrifuge the lysate at 10,000 × *g* in a microcentrifuge for 5 minutes. If a pellet has not formed by the end of the centrifugation, centrifuge an additional 15 minutes. Proceed immediately to Section IV.A or B.

III.B. From 3–5ml of Bacterial Culture

1. Pellet 3–5ml of cells by centrifugation at 10,000 × *g* for 10 minutes. Pour off the supernatant and blot the tube upside-down on a paper towel to remove excess media.
2. Completely resuspend the cell pellet in 300µl of Cell Resuspension Solution. Transfer the resuspended cells to a 1.5ml microcentrifuge tube.
3. Add 300µl of Cell Lysis Solution and mix by inverting the tube 4 times. The cell suspension should clear immediately.
4. Add 300µl of Neutralization Solution and mix by inverting the tube several times.

   Alternatively, if using an EndA+ strain, add 600µl of Neutralization Solution, mix by inverting the tube 4 times and incubate the lysate at room temperature for 10 minutes.

   Note: Some bacterial strains are more resistant to lysis and may require incubation for 3–5 minutes for efficient lysis.
5. Centrifuge the lysate at 10,000 × *g* in a microcentrifuge for 5 minutes. If a pellet has not formed by the end of the centrifugation, centrifuge an additional 15 minutes. Proceed immediately to Section IV.A or B.

III.C. From 5–10ml of Bacterial Culture

1. Pellet 5–10ml of cells by centrifugation at 1,400 × *g* for 10 minutes. Pour off the supernatant and blot the tube upside-down on a paper towel to remove excess media.
2. Completely resuspend the cell pellet in 400µl of Cell Resuspension Solution. Transfer the resuspended cells to a 1.5ml microcentrifuge tube.
3. Add 400µl of Cell Lysis Solution and mix by inverting the tube 4 times. The cell suspension should clear immediately.
4. Add 400µl of Neutralization Solution and mix by inverting the tube several times.

   Alternatively, if using an EndA+ strain, add 800µl of Neutralization Solution, mix by inverting the tube 4 times and incubate at room temperature for 10 minutes.

   Note: Some bacterial strains are more resistant to lysis and may require incubation for 3–5 minutes for efficient lysis.
5. Centrifuge the lysate at 10,000 × *g* in a microcentrifuge for 5 minutes. If a pellet has not formed by the end of the centrifugation, centrifuge an additional 15 minutes. Proceed immediately to Section IV.A or B.

IV. Plasmid Purification

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

- vacuum pump or vacuum aspirator capable of achieving a vacuum of 15–18 inches of Hg
- vacuum manifold (e.g., Vac-Man® or Vac-Man® Jr. Vacuum Manifold, Cat.# A7231, A7660)
- ethanol (95%, added as in Table 1)
- Nuclease-Free Water (Cat.# P1193)
- **optional:** 40% isopropanol/4.2M guanidine hydrochloride solution (required for EndA+ strains; use only Promega Cat.# H5381 or Amresco Cat.# 0118 guanidine-HCl)
- **optional (Section IV.B):** one 3ml disposable Luer-Lok® syringe per miniprep (e.g., Becton, Dickinson and Company Cat.# 9585)

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

IV.A. Plasmid Purification Using a Vacuum Manifold

Multiple Wizard® *Plus* Minipreps can be easily processed simultaneously with the Vac-Man® or Vac-Man® Jr. Laboratory Vacuum Manifold. For each miniprep, prepare one Wizard® Miniprep Column. Attach one of the Syringe Barrels to the Luer-Lok® extension of each Minicolumn. Insert the tip of the Minicolumn/Syringe Barrel assembly into the vacuum manifold. When all the columns are prepared, **close all of the stopcocks.**

1. Pipet 1ml of the resuspended resin into each barrel of the Minicolumn/syringe assembly. (If crystals or aggregates are present, dissolve by warming the resin to 25–37°C for 10 minutes. Cool to 30°C before use.)

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

2. Carefully remove all of the cleared lysate from each miniprep (supernatant from Step 5 of Section III.A, B or C) and transfer it to the barrel of the Minicolumn/syringe assembly containing the resin. No mixing is required.

   The resin and lysate should be in contact **only** for the time it takes to load the Minicolumns.

3. Open the stopcocks and apply a vacuum of at least 15 inches of Hg to pull the resin/lysate mix into the Minicolumn. When all of the sample has completely passed through the column, break the vacuum at the source.

   If using an EndA+ strain, add 2ml of 40% isopropanol/4.2M guanidine hydrochloride solution (see Section VII) to each column. Apply a vacuum and continue it for 30 seconds after all of the solution has flowed through the columns. Note that this solution will flow through the column more slowly than the standard Column Wash Solution. After this wash proceed with the standard column wash procedure (Step 4).

4. Add 2ml of the Column Wash Solution (containing 95% ethanol) to the Syringe Barrel and reapply the vacuum to draw the solution through the Minicolumn.

5. Dry the resin by continuing to draw a vacuum for 30 seconds after the solution has been pulled through the column. **Do not dry the resin for more than 30 seconds.** Remove the Syringe Barrel and transfer the Minicolumn to a 1.5ml microcentrifuge tube.

   Centrifuge the Minicolumn at 10,000 × *g* in a microcentrifuge for 2 minutes to remove any residual Column Wash Solution.

6. Transfer the Minicolumn to a new microcentrifuge tube. Add 50µl of nuclease-free water to the Minicolumn and wait 1 minute. Centrifuge the tube at 10,000 × *g* in a microcentrifuge for 20 seconds to elute the DNA.

   The DNA will remain intact on the Minicolumn for up to 30 minutes; however, prompt elution will minimize nicking of plasmids in the range of 20kb.

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.

7. Remove and discard the Minicolumn. 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 5µl of 10X TE buffer to the 50µl of eluted DNA.

IV.B. Plasmid Purification Without a Vacuum Manifold

It is possible to purify plasmid DNA using a 3ml syringe; however, the use of a vacuum source is more reproducible and generally results in higher quality DNA. If small numbers of samples are being processed we recommend the Vac-Man® Jr. Laboratory Vacuum Manifold. (A free Vac-Man® Jr. Laboratory Vacuum Manifold can be obtained by contacting Promega Corporation.)

1. For each miniprep, prepare one Wizard® Minicolumn. Remove the plunger from a 3ml disposable syringe (Becton, Dickinson and Company Cat.# 9585) and set it aside. Attach the syringe barrel to the Luer-Lok® extension of the Minicolumn and pipet 1ml of the resuspended resin into the barrel. (If crystals or aggregates are present, dissolve by warming the resin to 25–37°C for 10 minutes. Cool to 30°C before use.)

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

2. Carefully remove all of the cleared lysate from each miniprep (supernatant from Step 5 of Section III.A, B or C) and transfer it to the barrel of the Minicolumn/syringe assembly containing the resin.

The resin and lysate should be in contact **only** for the time it takes to load the Minicolumns.

3. Carefully insert the syringe plunger and gently push the slurry into the Minicolumn.

   If using an EndA+ strain, detach the syringe from the Minicolumn and remove the plunger from the syringe. Reattach the syringe barrel to the Minicolumn. Pipet 2ml of the 40% isopropanol/4.2M guanidine hydrochloride solution (Section VII) into the syringe barrel. Insert the syringe plunger into the syringe and gently push the solution through the Minicolumn. Note that this solution will flow through the column more slowly than the standard Column Wash Solution. After this wash proceed with the standard column wash procedure (Step 4).

4. Detach the syringe from the Minicolumn and remove the plunger from the syringe barrel. Reattach the syringe barrel to the Minicolumn.

   Pipet 2ml of Column Wash Solution (after the addition of ethanol) into the barrel of the Minicolumn/syringe assembly. Insert the plunger into the syringe and gently push the Column Wash Solution through the Minicolumn.

5. Remove the syringe and transfer the Minicolumn to a 1.5ml microcentrifuge tube. Centrifuge the Minicolumn at 10,000 × *g* in a microcentrifuge for 2 minutes to dry the resin.

6. Transfer the Minicolumn to a new 1.5ml microcentrifuge tube. Add 50µl of nuclease-free water to the Minicolumn and wait 1 minute. Centrifuge at 10,000 × *g* in a microcentrifuge for 20 seconds to elute the DNA.

   The DNA will remain intact on the Minicolumn for up to 30 minutes; however, prompt elution will minimize nicking of plasmids in the range of 20kb.

   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.

7. Remove and discard the Minicolumn. Follow these storage recommendations: DNA is stable in water without addition of a 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 5µl of 10X TE buffer to the 50µl of eluted DNA.

V. Supplementary Information

Plasmid DNA can be purified from 1–10ml overnight cultures of *E. coli* with the Wizard® *Plus* Minipreps 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.*

V.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.

V.B. Choosing a Bacterial Strain

Endonuclease I is a 12kDa periplasmic protein that degrades double-stranded DNA. This protein is encoded by the gene *endA*. The *E. coli* genotype *endA1* refers to a mutation in the wildtype *endA* gene, which produces an inactive form of the nuclease. *E. coli* strains with this mutation in the *endA* gene are referred to as EndA negative (EndA–). Table 2 contains a list of EndA– and EndA+ *E. coli* strains. The wildtype is indicated as EndA+. Using Wizard® *Plus* Minipreps, 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 in the protocols) 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.

Table 2. 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

V.C. Special Considerations for Automated Fluorescent Sequencing

For automated fluorescent sequencing applications, 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 DNA 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* Minipreps DNA Purification System routinely yields 4.0µg of plasmid DNA when used with the pGEM® Vector and DH5α™ cells in 1.5ml of LB medium. For low-copy-number plasmids, a larger culture volume

is required to obtain sufficient DNA for sequencing. Typical low-copy-number plasmid yields are 1.5–3.0µg of plasmid DNA from 10ml of LB culture medium when using pALTER®-1 (ampicillin-resistant) Vector and DH5α™ cells.

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.

VI. 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. Use only recommended culture volumes for low- and high-copy-number plasmids (Section III). 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.

VI. Troubleshooting (continued)

Symptoms	Causes and Comments
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 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.
	Low-copy-number plasmid used. Know the copy number of the plasmid used. Follow specialized instructions for use of low-copy-number plasmids. 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/or 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 that 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.

VI. Troubleshooting (continued)

Symptoms	Causes and Comments
Nicking of plasmid DNA	Overincubation during the alkaline lysis step. Total incubation of cell suspension with Cell Lysis Solution should not exceed 5 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. (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.
	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 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 III and IV) 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 DNA by agarose gel/ethidium bromide electrophoresis for more accurate quantitation.

VII. 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 95% ethanol as described in Table 1. Final ethanol concentration will be approximately 55%. (Components are listed at final concentration after ethanol addition.)

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.

VIII. Related Products

Product	Size	Cat.#
Wizard® *Plus* Maxipreps DNA Purification System*	10 preps	A7270
Wizard® *Plus* Megapreps DNA Purification System*	5 preps	A7300
Wizard® *Plus* Midipreps DNA Purification System*	25 preps	A7640
Wizard® *Plus* SV Minipreps DNA Purification System*	50 preps	A1330
Wizard® *Plus* SV Minipreps DNA Purification System + Vacuum Adapters*	50 preps	A1340
Wizard® *Plus* SV Minipreps DNA Purification System*	250 preps	A1460
Wizard® *Plus* SV Minipreps DNA Purification System + Vacuum Adapters*	250 preps	A1470
Wizard® Midipreps DNA Purification Resin*	1,000ml	A7701
Wizard® Maxipreps DNA Purification Resin*	500ml	A7401
Wizard® Megapreps DNA Purification Resin*	1,000ml	A7361
Wizard® Midicolumns	100 each	A7651
Wizard® Maxi/Megapreps Filtering System	50 each	A7421
Vac-Man® Laboratory Vacuum Manifold	20-sample capacity	A7231
Vac-Man® Jr. Laboratory Vacuum Manifold	2-sample capacity	A7660
Cell Resuspension Solution*	150ml	A7112
Cell Lysis Solution* (Plasmid Purification)	150ml	A7122
Neutralization Solution*	150ml	A7131
Column Wash Solution*	125ml	A8102
One-Way Luer-Lok® Stopcocks	10 each	A7261

*For Laboratory Use.

IX. 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.

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[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.

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