Technical Bulletin

# Wizard® MagneSil® Plasmid Purification System

INSTRUCTIONS FOR USE OF PRODUCTS A1630, A1631, A1635, A1641 AND A2201.

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# Wizard® MagneSil® Plasmid Purification System

**All technical literature is available on the Internet at www.promega.com/tbs Please visit the web site to verify that you are using the most current version of this Technical Bulletin. Please contact Promega Technical Services if you have questions on use of this system. E-mail techserv@promega.com.**



## 1. Description

The Wizard® MagneSil® Plasmid DNA Purification System[a,b] provides a simple and reliable method for the rapid isolation of plasmid DNA in a multiwell format. The purified plasmid can be used directly for automated fluorescent DNA sequencing, such as with BigDye® terminator sequencing chemistry, as well as for other standard molecular biology techniques including restriction enzyme digestion.

The use of paramagnetic particles for lysate clearing as well as DNA capture circumvents the need for centrifugation or vacuum manifolds, making the system ideal for full automation.

The Wizard® MagneSil® Plasmid Purification system uses alkaline SDS-lysis to generate the bacterial lysate and incorporates MagneSil® Paramagnetic Particles[b,c] for both lysate clearing and plasmid purification. The procedure is performed using a number of simple steps:

- Alkaline lysis of bacterial cell pellets
- Lysate clearing using MagneSil® BLUE[c]
- Plasmid capture on MagneSil® RED[c]
- Washing with 80% ethanol
- Elution

### Selected Citations using the Wizard® MagneSil® Plasmid Purification System

- Klein, P.E., Klein, R.R., Vrebalov, J. and Mullet, J.E. (2003) Sequence-based alignment of sorghum chromosome 3 and rice chromosome 1 reveals extensive conservation of gene order and one major chromosomal rearrangement. *Plant J.* **34**, 605–621.

  Overnight cultures were used for plasmid purification using the Wizard® MagneSil® Plasmid Purification System and a Beckman Coulter Biomek® 2000 automated laboratory workstation. The purified plasmids were sequenced with plasmid-specific primers, and the sequence was compared to the complete rice genome to map similarities.

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

## 2. Product Components

| Product | Size | Cat.# |
|---|---|---|
| Wizard® MagneSil® Plasmid Purification System | 4 × 96 | A1630 |

Each system includes sufficient reagents for 4 × 96-well plates. Includes:

- 50ml Cell Resuspension Solution
- 60ml Cell Lysis Solution
- 60ml Neutralization Solution
- 19ml MagneSil® BLUE
- 30ml MagneSil® RED
- 50ml Elution Buffer
- 16 Collection Plates

| Product | Size | Cat.# |
|---|---|---|
| Wizard® MagneSil® Plasmid Purification System | 8 × 96 | A1631 |

Each system includes sufficient reagents for 8 × 96-well plates. Includes:

- 90ml Cell Resuspension Solution
- 125ml Cell Lysis Solution
- 120ml Neutralization Solution
- 38ml MagneSil® BLUE
- 60ml MagneSil® RED
- 100ml Elution Buffer
- 32 Collection Plates

| Product | Size | Cat.# |
|---|---|---|
| Wizard® MagneSil® Plasmid Purification System, HTP1 | 100 × 96 | A1635 |

The HTP1 system provides sufficient reagents to process 100 × 96 well plates when performed on a Beckman Coulter Biomek® workstation using a standard configuration. Includes:

- 3 × 500ml Cell Resuspension Solution
- 3 × 500ml Cell Lysis Solution
- 3 × 500ml Neutralization Solution
- 5 × 100ml MagneSil® BLUE
- 7 × 100ml MagneSil® RED
- 3 × 500ml Elution Buffer
- 100 Collection Plates (4-pack)

### Items Available Separately

| Product | Size | Cat.# |
|---|---|---|
| MagneSil® BLUE | 100ml | A2201 |
| MagneSil® RED | 100ml | A1641 |
| Cell Resuspension Solution | 500ml | A7114 |
| Cell Lysis Solution | 500ml | A7124 |
| Neutralization Solution | 500ml | A7132 |
| Elution Buffer | 500ml | A1655 |

**Storage Conditions:** Store all Wizard® MagneSil® Plasmid Purification System components at 22–25°C.

## 3. System Requirements

This protocol requires a magnetic workstation that will accommodate a 96-well microtiter plate. The MagnaBot® 96 Magnetic Separation Device (Cat.# V8151) can be used. The protocol also requires an orbital multiwell plate shaker. This protocol has been optimized using the Micro Mix 5 Shaker (Section IX.B).

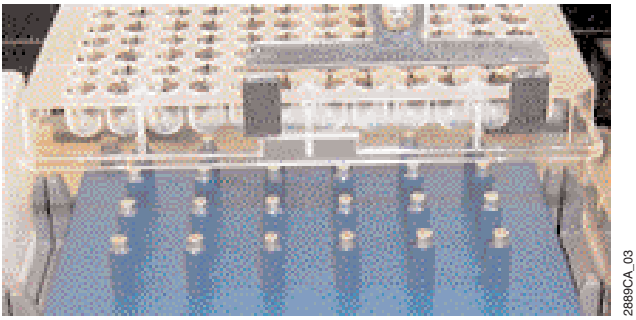



**Figure 1. MagnaBot® 96 Magnetic Separation Device.**

## 4. Manual Protocol for Plasmid DNA Isolation and Purification

### Materials to be Supplied by the User

(Solution compositions are provided in Section IX.A.)

- Culture medium containing appropriate antibiotic
- Tabletop centrifuge capable of 1,500 × *g*, fitted with 96-well plate adapters (e.g., tabletop model or Beckman J2HC model #362701 centrifuge)
- MagnaBot® 96 Magnetic Separation Device (Cat.# V8151)
- Microplate shaker fitted with plate holders (We recommend Micro Mix 5 Shaker plus custom shaker integration kit C5016 MM5kit, ACME Automation, PO Box 1119, Spring City, TN 37381)
- Optional: Multichannel pipettors capable of dispensing 10–1,000µl
- Two plate holders for shaker (labware holder, gray, Beckman# 609120)

### To process one plate of 96 samples

- Deep-well (2ml) 96-well plate (e.g., Beckman deep-well titer plate, item #140504)
- 4 boxes of 96 disposable P200 tips, sterile or nonsterile
- 30ml 80% ethanol
- 1 plate sealer

### 4.A. Equipment Setup

Set up the microplate shaker with orbital action such that cell pellets are completely resuspended and that mixing of the particles with lysates achieves maximum binding of DNA to the particles. The shaker optimizes the process to achieve the maximum yield and increases the robustness of the process. The suggested settings included in this protocol refer specifically to the Micro Mix 5 orbital shaker with fitted platform and plate holders (C5016 MM5 kit, ACME Automation, www.acme-automation.com). **If an alternate shaker is used, settings must be empirically determined by the user.** See Section 9.B for additional information.

### 4.B. Preparation of Cell Pellets and Cell Resuspension

1. Pellet the bacterial culture grown in a 2ml deep-well culture plate with square wells (e.g., Beckman deep-well titer plate, item# 140504) by centrifuging for 15 minutes at 1,500 × *g* in a tabletop centrifuge. Cells should contain high copy number plasmids. For additional information on choosing a bacterial strain, see Section 9.C. As much as 6.0 $O.D._{600}$ of total cell mass may be processed per well. Pour off the supernatant and blot the plate upside down on a paper towel to remove excess liquid.

   **Note:** Do not attempt to process more than 6.0 $O.D._{600}$ of total cell mass. Cells pellets may be stored at –20°C; however, storage for more than 30 days is not recommended.

   Place the 96-well, deep-well plate with cell pellets on the shaker in a plate holder that clamps the plate to the shaker platform.

2. Add 90µl of Cell Resuspension Solution to each well of the deep-well plate. Shake using the following settings: Form 20, Amplitude 8 for 5 minutes.

### 4.C. Cell Lysis and Lysate Clearing

1. Add 120µl of Cell Lysis Solution to each well of the deep-well culture plate. Resuspend cells by shaking using the following settings: Form 39, Amplitude 6 for 3 minutes.

2. Add 120µl of Neutralization Solution to each well of the deep-well culture plate to neutralize the lysate. Shake using the following settings: Form 20, Amplitude 7 for 3 minutes. A flat, floating precipitate should form in each well.

3. Add 25µl MagneSil® BLUE to each well of the deep-well culture plate. Shake using the following settings: Form 20, Amplitude 8 for 1 minute.

   **Note:** Shake the resin bottle vigorously and thoroughly immediately BEFORE removing any material.

4. Transfer 300µl of the neutralized lysate containing the MagneSil® BLUE particles from each well of the deep-well plate to the wells of a Collection Plate (for clearing) that is sitting on a MagnaBot® 96 Magnetic Separation Device.

5. Allow 90 seconds on the MagnaBot® 96 Magnetic Separation Device for magnetized pellets to form.

### 4.D. DNA Binding

**Note:** If you are using the suggested shaker settings, do not remove the deep-well plate from the shaker. It is important to maintain this mass.

1. Prepare a Collection Plate for binding by adding 25µl MagneSil® RED to each well of the plate.
2. Transfer 120µl of the cleared lysate from each well of the Collection Plate used for clearing to the wells of the Collection Plate prepared for binding in Step D1. Place the Collection Plate for binding on the shaker.
3. Perform the first binding mix using the following settings: Form 47, Amplitude 6 for 2 minutes. **Note:** Amplitude can vary between instruments and may need to be adjusted to avoid splashout yet provide adequate mixing.
4. Set aside the Collection Plate for Clearing. Transfer Collection Plate for binding to the MagnaBot® 96 Magnetic Separation Device, and allow pellets to form. Discard the spent liquid from each well.
5. Transfer the Collection Plate for binding to the shaker, and add an additional 25µl of MagneSil® RED to each well of the plate.
6. Place the Collection Plate containing the remaining cleared lysate on the MagnaBot® 96 Magnetic Separation Device. Transfer remaining 120µl of cleared lysate from each well to the wells of the binding plate.
7. Perform second binding mix in the Collection Plate for binding on the shaker using the following settings: Form 47, Amplitude 6 for 2 minutes.

   **Note:** Amplitude can vary between instruments and may need to be adjusted.
8. Discard the Collection Plate that was used for clearing the lysate.
9. Transfer the Collection Plate for binding to the MagnaBot® 96 Magnetic Separation Device, and allow pellets to form. Discard the spent liquid from each well.

### 4.E. Washing

1. Add 100µl of 80% ethanol to Collection Plate for binding and transfer it to the shaker. Resuspend the resin by shaking using the following settings: Form 47, Amplitude 4 for 1 minute.
2. Transfer the Collection Plate for binding to MagnaBot® 96 Magnetic Separation Device, and allow pellets to form. Discard the spent wash.
3. Transfer the Collection Plate for binding to the shaker, and repeat above wash process 2 times for a total of three washes.

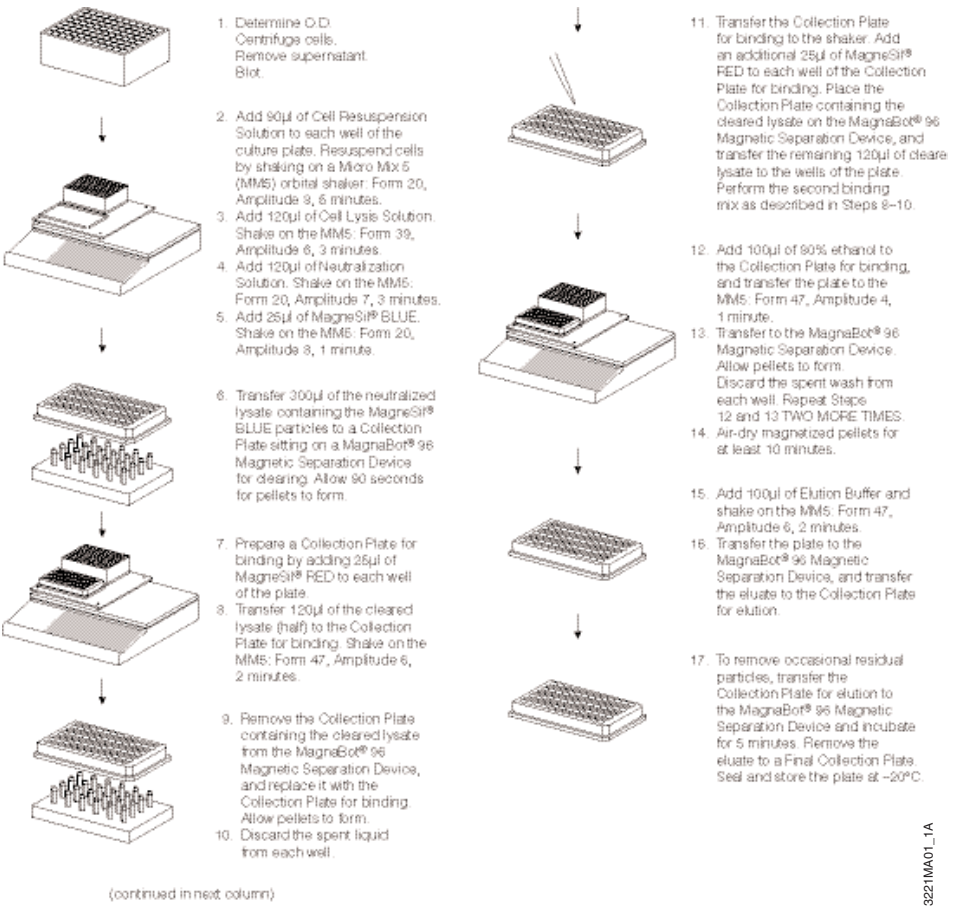
### 4.F. Drying

1. Allow the magnetized pellets in Collection Plate for binding to air-dry for at least 10 minutes. **Note:** Samples may be dried for several hours with no adverse effects.
2. Transfer the Collection Plate for binding to the shaker.

### 4.G. Elution of DNA

1. Add 100µl of Elution Buffer and shake using the following settings: Form 47, Amplitude 6 for 2 minutes.
2. Transfer the Collection Plate for binding to the MagnaBot® 96 Magnetic Separation Device, and transfer eluate to the Collection Plate for elution.

   **Note:** Eluate volumes may vary but are generally at 80–90µl.
3. To remove occasional residual particles, transfer Collection Plate for elution to the MagnaBot® 96 Magnetic Separation Device, and allow to stand for 5 minutes. After 5 minutes, remove the eluate and transfer it to the Final Collection Plate.
4. Seal the plate and store at –20°C.




(continued in next column)

**Figure 2. Protocol for manual plasmid DNA purification using the Wizard® MagneSil® Plasmid Purification System and the Micro Mix 5 Shaker (Acme Automation). Note:** Amplitude can vary between instruments and may need to be adjusted to avoid splashout, yet provide adequate mixing.

## 5. Use with Robotic Workstations

The manual protocol described in Section 4 can be used as a guide to develop protocols for automated workstations. The protocol may require optimization depending on the instrument used.

Promega has an ongoing effort to adapt this procedure to other platforms. This system has been fully automated on the Beckman Biomek® 2000. Downloadable methods are available on the Internet at: www.promega.com/automethods/

As new methods are developed, they will be posted to the Promega web site.

## 6. Preparation of *E. coli* Cultures for Plasmid Isolation

Dispense 0.5 to 1.5ml of culture medium containing antibiotic into the wells of the 96-well culture plate. Choose a single, well-isolated colony from a fresh agar plate containing the same antibiotic to inoculate each plate well. The inoculated cultures should be incubated overnight (16–17 hours at 37°C) with agitation. CIRCLEGROW® medium is recommended for growth of *E. coli* host to obtain maximum cell biomass. An $O.D._{600}$ of 1.0–6.0 for high-copy number plasmids ensures that bacteria have reached the proper growth density for harvesting and plasmid DNA isolation. Using cultures that have $O.D._{600}$ readings >6.0 may lead to incomplete processing of the bacterial lysate. This may decrease yields as well as increase contaminant levels in the isolated plasmid DNA.

**Note:** The culture volume may vary to equal a maximum $O.D._{600}$ of 6.0 per well. It is not critical to determine the $O.D._{600}$ unless there is a possibility that the total cell mass may exceed an $O.D._{600}$ value of 6.0 per well. The recommended minimum total cell mass to process per well is an $O.D._{600}$ value of 1.0. The biomass of cultures will vary depending on culture media used, growth time and temperature, agitation speed, host strain used and nature of the plasmid insert.

## 7. Troubleshooting

| Symptoms | Causes and Comments |
|---|---|
| Incomplete resuspension of cells (cell pellet still visible) | Cells stored too long at –20°C. Cells should only be stored 2–6 weeks, maximum, at –20°C. Shake an additional 5 minutes. |
| | Too many cells were used (biomass greater than 6.0 $O.D._{600}$). Do not attempt to process more than 6.0 $O.D._{600}$ of total cell mass per well. |
| | Cells may not be thawed. Insure frozen cells are thawed completely by allowing the cells to sit at room temperature at least 15 minutes. |
| Turbidity observed in cleared lysate | Too long at neutralization step. DO NOT shake longer than 4 minutes. The lysate will remain cloudy, and decreased plasmid DNA yield will result. |
| | Lysis may be incomplete. Make sure frozen cells are completely thawed. Increase lysis time from 3 to 5 minutes. |
| | Too many cells were used (biomass greater than 6.0 $O.D._{600}$). Increase lysis time from 3 to 5 minutes. Do not attempt to process more than 6.0 $O.D._{600}$ per well. |
| Flat floating precipitate does not form during lysate neutralization step within 4 minutes. | Incorrect reagent may have been added. Check reagent source or trough. |
| Compact pellet does not form at magnet corner, and complete removal of cleared lysate is not possible. | Too many cells were used (biomass greater than 6.0 $O.D._{600}$). Do not attempt to process more than 6.0 $O.D._{600}$ of total cell mass. |
| | Growth medium may be interfering with the protocol. We recommend CIRCLEGROW® medium for this protocol. |
| | Magnetic flux may be insufficient. Use only the Collection Plates provided with this system or plates of the same design. |
| Resin and lysate thrown from well during mixing | Too much lysate was transferred to well. The protocol requires the binding to occur in two steps of 120µl. This approach avoids the use of a deep-well plate at this step. |
| | Shaker may be set incorrectly. Check shaker setting. Try different **amplitudes** to eliminate splashing. |

## 7. Troubleshooting (continued)

| Symptoms | Causes and Comments |
|---|---|
| Resin and lysate thrown from well during mixing (continued) | Incorrect plates were used. Use only the Collection Plates provided with this system or plates of the same design. |
| | Plate clamp may not be holding the plate tightly. There should be no play when the plate is clamped. **CAUTION:** If robotic arm or gripper is used, be sure to adjust the clamp tightness so the gripper can remove the plate during the process. |
| Downstream applications are problematic | Insufficient washing of the magnetic particles. Thorough washing is required to remove salts that might interfere with downstream applications. Insure that the magnetic particles are thoroughly suspended in the well by the action of the shaker at each wash step. |
| | Alcohol may have been carried over. Insure that all the spent wash is removed from the well. If alcohol is spilled while shaking, lessen the amplitude. Allow magnetic pellet to air-dry for at least 10 minutes. Samples can be dried for several hours with no adverse effects. |
| Low DNA yield or eluate volumes | Particles not completely resuspended. Thoroughly resuspend the particles after adding the elution buffer. |
| | The elution buffer may have spilled during mixing. Lessen the amplitude of the shaking. |
| | The binding resin and lysate may not have been mixed thoroughly. The binding resin and lysate must be thoroughly mixed to insure maximum binding. |
| Particle carryover in final elution plate | Aspiration rate may be too high. Reduce the aspiration rate. |
| | Particles may be adhering to the tip walls. If you are reusing tips or using fixed tips, rinse them to remove adherent particles. Add a second magnetic step to remove particles. |

## 8. Related Products

### Wizard® SV 96 Plasmid Purification Systems

| Product | Size | Cat.# |
|---|---|---|
| Wizard® SV 96 Plasmid DNA Purification System* | 1 × 96 preps | A2250 |
| | 5 × 96 preps | A2255 |
| Wizard® SV 96 Cell Resuspension Solution* | 500ml | A7113 |
| Wizard® SV 96 Cell Lysis Solution* | 500ml | A7123 |
| Wizard® SV 96 Neutralization Solution* | 500ml | A1481 |
| Wizard® SV Wash Solution* | 185ml | A1311 |
| Binding Plates* | 10 pack | A2271 |
| Wizard® SV 96 Lysate Clearing Plates* | 10 pack | A2241 |
| Vac-Man® Vacuum Manifold | 96-well capacity | A2291 |

*For Laboratory Use.

| Product | Size | Cat.# |
|---|---|---|
| Wizard MagneSil Tfx™ System | 4 × 96 preps | A2380 |
| | 8 × 96 preps | A2381 |
| MagnaBot® 96 Magnetic Separation Device | 1 each | V8151 |

## 9. Appendix

### 9.A. 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 |
| 1.0% | SDS |

**Neutralization Solution**

| | |
|---|---|
| 1.32M | potassium acetate |

(Final pH is 4.8)

**Elution Buffer**

| | |
|---|---|
| 10mM | Tris-HCl (pH 8.5) |

**80% ethanol wash solution**

Prepare 100ml of wash solution of 80% ethanol by adding 80ml of 100% ethanol to 20ml of high-quality water (or 84ml 95% ethanol to 16ml of high-quality water). This can be stored at 20–25°C.

The user will need 33ml/reservoir per 96-well plate processed.

**CIRCLEGROW® culture medium** can be purchased from Qbiogene, 2251 Rutherford Road, Carlsbad, CA 92008.

### 9.B. Microplate Shaker Considerations

We have found that the efficiency and type of mixing achieved by the shaker used in this protocol is a critical factor in achieving the maximum yield and quality of plasmid DNA.

We recommend the microplate shaker MM5 with custom shaker integration kit for robotic platforms as supplied by ACME Automation (www.acme-automation.com). This kit contains the shaker, custom platform, plate clips, serial cable and software drivers required to integrate the shaker with instrumentation software.

This shaker is fully programmable and allows complete control over the amplitude, frequency, direction of rotation and duration of shaking. The shaker has capacity for four 96-well microplates. ACME supplies a custom integration kit for the Tecan Genesis® and the Beckman Coulter Biomek® 2000 workstation. This shaker may be adapted for use with other instrument platforms.

### 9.C. 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 *endA* gene that results in the production of an inactive form of the nuclease. *E. coli* strains with this mutation in the *endA* gene are referred to as EndA negative (EndA–). Table one contains a list of EndA– and EndA+ *E. coli* strains.

**Table 1. EndA– and EndA+ *E. coli* strains.**

| | | | |
|---|---|---|---|
| **EndA– Strains:** | | | |
| BJ5183 | DH1 | DH20 | DH21 |
| DH5α™ | JM103 | JM105 | JM106 |
| JM107 | JM108 | JM109 | MM294 |
| SK1590 | SKI592 | SK2267 | SRB |
| XL1-Blue | XLO | | |
| **EndA+ Strains:** | | | |
| BL21(DE3) | CJ236 | HB101 | JM83 |
| LE392 | MC1061 | NM522* | NM554* |
| P2392 | PR700* | Q358 | RR1 |
| TB1 | TG1 | Y1088* | BMH71-18 |
| ES1301 | | | |

* All NM, PR and Y10 series are EndA+.

### 9.D. Calculations

An O.D.$_{600}$ of total cell biomass is defined as 10X O.D.$_{600}$ per 1ml when using a 1:10 dilution of the culture measured in a 1cm path length cuvette.

### 9.E. Suggested Reagent Volumes in Reagent Troughs for Manual Dispensing with a Multichannel Pipettor

| Reagent | Volume per well | Volume per 96-well plate | Volume per reservoir to process one plate |
|---|---|---|---|
| Cell Resuspension Solution | 90.0µl | 8.7ml | 10.0ml |
| Cell Lysis Solution | 120.0µl | 11.6ml | 13.0ml |
| Neutralization Solution | 120.0µl | 11.6ml | 13.0ml |
| MagneSil® BLUE | 25.0µl | 2.4ml | 3.5ml |
| MagneSil® RED | 50.0µl | 4.8ml | 6.0ml |
| Elution Buffer | 100.0µl | 9.6ml | 11.0ml |
| 80% ethanol (not supplied) | 300.0µl | 28.8ml | 33.0ml |

### 9.F. Special Considerations for Automated Fluorescent Sequencing

For applications such as fluorescent DNA sequencing, special considerations should be given to the selection of plasmid and *E. coli* strains to optimize yield and plasmid quality. Optimal automated fluorescent sequencing results are obtained by using high-copy number plasmids and EndA– strains of *E. coli* for plasmid propagation.

Purified plasmid DNA must be within the proper concentration range for successful automated cycle sequencing (ideally 100ng/µl and not less than 40ng/µl). Concentrations achieved with high-copy number plasmid DNA purified using the Wizard® MagneSil® System are of sufficient concentration for direct use in these applications. The yields range from 60ng/µl to 100ng/µl. We 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 may require a large amount of sample.

### 9.G. Special Considerations for Sequencing Using BigDye® Chemistry

If the BigDye® terminator ready reaction mix is diluted, it is essential to use an appropriate dilution buffer, such as DNA 5X sequencing buffer (250mM Tris-HCl [pH 9.0 at 25°C], 10mM $MgCl_2$).

[a]U.S. Pat. Nos. 6,027,945, 6,368,800 and 6,673,631, Australian Pat. No. 732756, European Pat. No. 1 204 741, Mexican Pat. No. 209436 and other patents pending.

[b]U.S. Pat. Nos. 6,284,470 and 7,078,224, Australian Pat. No. 778486 and other patents pending.

[c]U.S. Pat. Nos. 6,027,945, 6,368,800 and 6,673,631, Australian Pat. No. 732756, Japanese Pat. No. 3253638, European Pat. No. 1 204 741, Mexican Pat. No. 209436 and other patents pending.



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All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.