Technical Bulletin

Wizard® MagneSil® Sequencing Reaction Clean-Up System

INSTRUCTIONS FOR USE OF PRODUCTS A1831, A1832, A1835 AND A8231.

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Wizard® MagneSil® Sequencing Reaction Clean-Up System

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1. Description

Automated fluorescent sequencing using the ABI PRISM® BigDye® Terminator chemistry is the method of choice for high-throughput DNA sequence determination. Sequence ladders are generated by the standard Sanger method (1) using fluorescently labeled chain terminator nucleotides (2). These BigDye® terminators are dideoxynucleotide triphosphates linked to an energy transfer dye composed of a fluorescein donor and one of four d-rhodamine-based acceptors (3). Automated sequencers separate the labeled sequence extension products using a molecular sieve matrix and identify the terminal nucleotide based on its emission wavelength.

If not removed prior to analysis, certain reaction components will interfere with data collection. Buffer salts give rise to aberrant electrophoretic migration, and unincorporated dye-labeled terminators and related species interfere with the legitimate signal from sequencing extension products. Common purification

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1. Description (continued)

methods based on gel filtration and precipitation by ethanol are problematic for high-throughput robotic applications because they can require multiple centrifugation and/or vacuum drying steps.

Promega has developed MagneSil® Paramagnetic Particles (PMPs) to purify nucleic acids such as plasmid DNA and PCR fragments. This technology also can be applied to the high-throughput purification of BigDye® terminator DNA sequencing reactions prior to automated sequence analysis. Procedures have been developed on several robotic workstations using the standard 96-well amplification plate format. Sequence quality is similar to or better than standard manual methods and offers the distinct advantage of a hands-off protocol. No user intervention is required from the time the 96-well amplification plate is put on the robot deck until samples are loaded onto the DNA sequencer.

Selected Citation Using the Wizard® MagneSil® Sequencing Reaction Clean-Up System:

Shirasawa, S. *et al.* (2004) SNPs in the promoter of a B cell-specific antisense transcript, SAS-ZFAT, determine susceptibility to autoimmune thyroid disease. *Hum. Mol. Gen.* **13,** 2221-31.

Autoimmune thyroid disease (AITD) is caused by an immune response to self-thyroid antigens and has a significant genetic component. Antisense RNA transcripts have been implicated in gene regulation. The authors identified a novel zinc-finger gene as one of the susceptibility genes in 8q23–q24 through an initial association analysis using the probands in the previous linkage analysis and a subsequent association analysis of the samples from a total of 515 affected individuals and 526 controls. Wizard® MagneSil® Sequencing Reaction Clean-Up System was used to clean amplication reaction products, which then were used in sequencing reactions.

For additional Wizard® MagneSil® Sequencing Reaction Clean-Up System citations, visit: **www.promega.com/citations/**

Note: The Wizard® MagneSil® Sequencing Reaction Clean-Up System[a] is designed to purify sequencing extension products generated from a variety of template DNAs. This system can be coupled with the Wizard® SV 96 (Cat.# A2250) and MagneSil® Plasmid DNA Purification Systems (Cat.# A1630).

2. Product Components and Storage Conditions

Product	Cat.#
Wizard® MagneSil® Sequencing Reaction Clean-Up System	A1831

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

- 1 × 100ml MagneSil® GREEN

Product	Cat.#
Wizard® MagneSil® Sequencing Reaction Clean-Up System	A1832

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

- 2 × 100ml MagneSil® GREEN

Product	Cat.#
Wizard® MagneSil® Sequencing Reaction Clean-Up System	A1835

Each system contains sufficient reagents for 100 × 96-well plates.
Includes:

- 25 × 100ml MagneSil® GREEN

Product	Size	Cat.#
MagneSil® GREEN[a,b]	100ml	A8231

Storage Conditions: Store all components at room temperature.

Do **not** freeze the product.

Sufficient MagneSil® GREEN is provided to clean up the stated number of 20µl sequencing reactions when performed manually or on the Beckman Coulter Biomek® 2000 or Biomek® FX automation workstation. Because of variations in the residual reservoir volumes, other automated platforms may require larger volumes of MagneSil® GREEN.

3. 96-Well Plate Procedure

The Wizard® MagneSil® Sequencing Reaction Clean-Up System purifies BigDye® terminator sequencing reaction extension products from other reaction components prior to analysis by automated sequencers. The protocol removes unincorporated dye-labeled terminators and exchanges the buffer for gel loading solution (Figure 1). For information on use with 384-well plates, see Section 5.

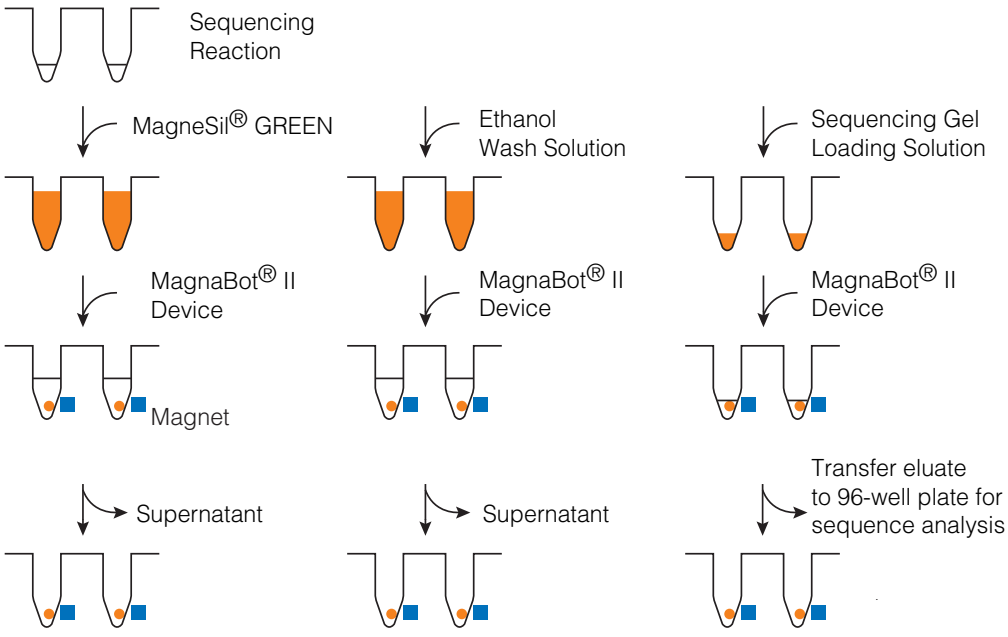
The system works with the ABI PRISM® 310 and 3100 Genetic Analyzers, ABI PRISM® 377 DNA Sequencer and ABI PRISM® 3700 DNA Analyzer. Sequence quality is assessed by accuracy (and/or PHRED score), read length and start position. The system is qualified to provide sequence data that are greater than 98% accurate over 600 bases when using the control template and primer included in the ABI PRISM® BigDye® terminator cycle sequencing reaction kits.

3.A. Sequencing Reaction Setup

Follow the most recent version of the protocol for the ABI PRISM® BigDye® terminator cycle sequencing ready reaction kit. Sample input for the Wizard® MagneSil® Sequencing Reaction Clean-Up System is assumed to be 20µl of sequencing reaction with either no dilution or a 1:4 dilution of the terminator ready reaction mix. If other sequencing reaction volumes are used, scale the volumes in the protocol appropriately.

If diluting the terminator ready reaction mix, maintain the buffering capacity at 50mM Tris-HCl (pH 9.0) and Mg^{2+} concentration at 2mM with $MgCl_2$. Acceptable results can be achieved when the terminator ready reaction mix is diluted up to fourfold (1/4X), while supplementing with DNA Sequencing 5X Buffer (250mM Tris-HCl [pH 9.0 at 25°C], 10mM $MgCl_2$). The following is an example of a sequencing reaction setup for plasmid DNA template.

	1X	**1/4X**
terminator ready reaction mix	8µl	2µl
DNA, primer and water	12µl	15µl
DNA Sequencing 5X Buffer	––	3µl
sequencing reaction total volume	**20µl**	**20µl**



Binding

The Binding Solution has been optimized so that the PMPs selectively bind the sequencing extension products. A magnet is used to capture and hold the PMPs against the side of the well. Binding Solution and nonbound sequencing reaction components are removed to waste.

Washing

Samples are removed from the magnet, and nonspecifically bound material is removed by washing with ethanol. The PMPs are resuspended in ethanol wash solution, mixed and captured. The wash solution containing nonspecifically bound terminators and residual salts are removed to waste. The wash is repeated for a total of two washes, then the samples are air-dried.

Elution

Sequencing extension products are eluted in gel loading solution. The particles are resuspended in the appropriate sequencing gel loading solution, then captured. Eluted sample is removed and ready to analyze.

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Figure 1. Schematic diagram of the Wizard® MagneSil® Sequencing Reaction Clean-Up System protocol.

3.B. Protocol

Materials to be Supplied by the User
(Solution compositions are provided in Section 6.)

- MagnaBot® II Magnetic Separation Device (Cat.# V8351)
- Plate Clamp 96 (Cat.# V8251; for unskirted 96-well plates)
- Plate Stand (Cat.# V8261; for use on automated platforms)
- 96-well plate (Robbins Scientific CyclePlate®-96ET, Cat.# 1055-00-D or USA Scientific TempPlate II, Cat.# 1402-9600)
- 90% ethanol wash solution
- elution/loading solution

This protocol is provided for customers performing a manual evaluation of this product or designing a protocol for an instrument for which we do not supply a protocol.

DNA Binding

1. Assemble the 96-well plate into the Plate Clamp 96 (Figure 2, Panels A and B).
2. Use the Plate Stand (Figure 2, Panel C) to position the plate on the robotic deck.
3. Resuspend the MagneSil® GREEN particles by vigorously shaking the bottle. Add 180µl of MagneSil® GREEN particles to each 20µl sequencing reaction.
4. Incubate at room temperature for 5 minutes. Mix by pipetting at 0, 2.5 and 5 minutes.
5. Place the plate onto the MagnaBot® II Magnetic Separation Device (Figure 2, Panel D) to capture the particles.
6. Remove and discard the liquid. Avoid removing any particles.

Washing

7. Remove the plate from the MagnaBot® II Device and place on Plate Stand.
8. Add 100µl of 90% ethanol to each sample.
9. Incubate at room temperature for 5 minutes. Mix by pipetting at 0, 2.5 and 5 minutes.
10. Place the plate on the MagnaBot® II Device to capture particles.
11. Remove and discard the liquid. Avoid removing any particles.
12. Repeat Steps 7–11 for a total of two washes.
13. Allow the particles to air-dry for approximately 10 minutes at room temperature.

Elution

14. Add appropriate elution/loading solution.

Instrument	Elution/Loading Solution	Volume
ABI PRISM® 377	formamide/blue dextran/EDTA (see Section 6)	6µl
ABI PRISM® 310	template suppression reagent*	10–20µl
ABI PRISM® 3100	Hi-Di™ formamide (Applied Biosystems Cat. #4311320)	10–20µl
ABI PRISM® 3700	Hi-Di™ formamide or water	10µl

*Supplied with Applied Biosystems POP-6 polymer.

15. Mix well by pipetting. Incubate at room temperature for 1–2 minutes.

16. Place the plate on the MagnaBot® II Device to capture particles.

17. Transfer purified sequencing reactions to a clean 96-well plate. Be careful to avoid removing any particles.

Note: It is not necessary to heat denature purified sequencing reactions.

4. Use With Robotic Workstations

4.A. Protocols

The generic protocol described in Section 3.B can be used as a guide to develop protocols for robotic workstations. Incubation times, solution delivery, removal times and other aspects of the protocol may require optimization, depending on the instrument used. For example, we have found that two ethanol washes in Step 12 of Section 3.B is sufficient for the manual protocol and some automated platforms, but the Biomek® 2000 and Biomek® FX methods require three ethanol washes for best results.

Promega has an ongoing effort to develop procedures for a variety of automated platforms. For information on an automated method for your system, go to: **www.promega.com/automethods/** and provide your contact information. An Automated Support Team member will contact you regarding a method for use with your particular system. Some methods can be downloaded directly from the Web site.

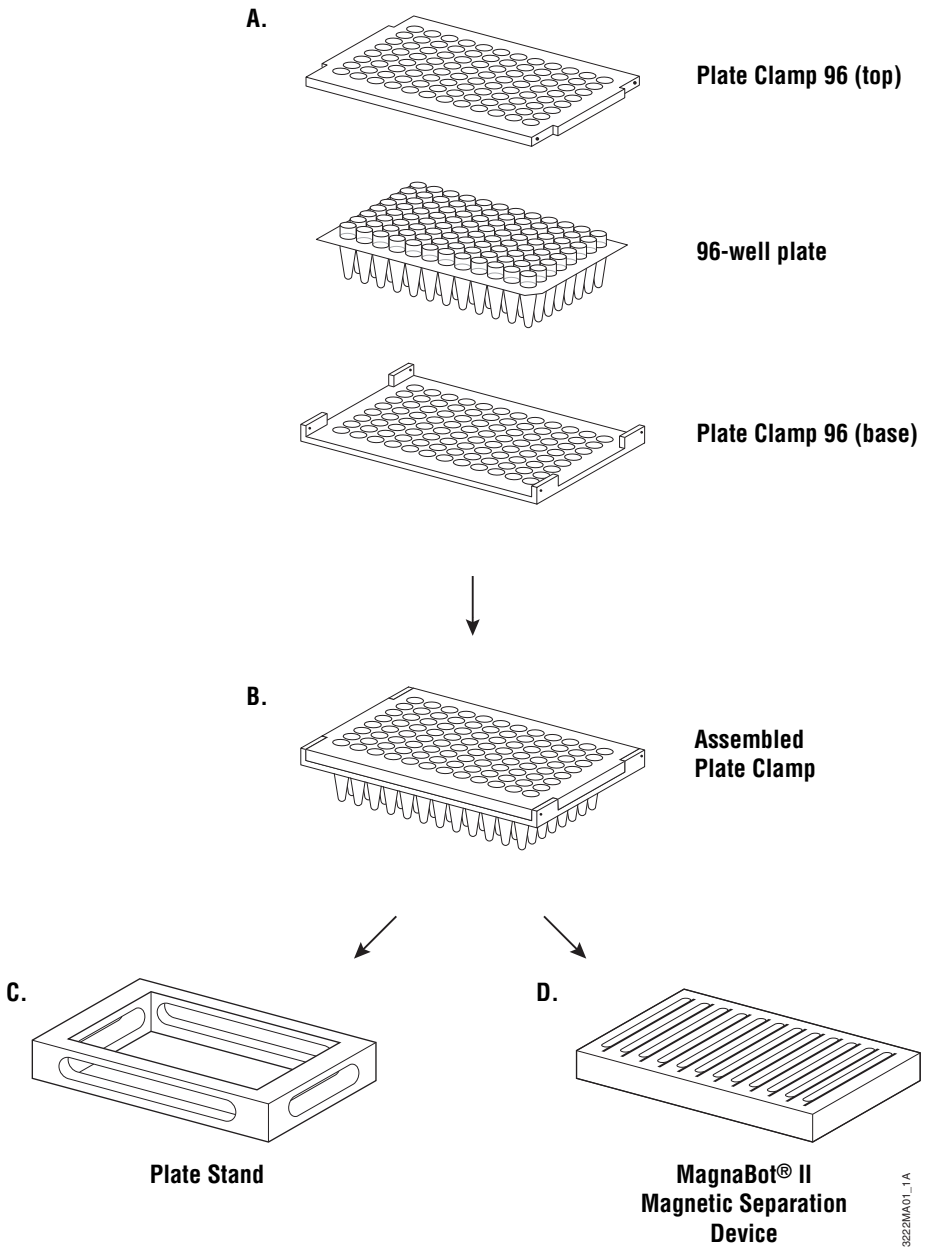

Figure 2. Accessories for the Wizard® MagneSil® Sequencing Reaction Clean-Up System. To eliminate plate warpage, the unskirted 96-well plate is placed into the Plate Clamp 96 (**Panels A and B**). During the protocol, the Plate Clamp 96 assembly can be held by the Plate Stand (**Panel C**) or placed onto the MagnaBot® II Device for magnetic separation of the MagneSil® Paramagnetic Particles (**Panel D**).

4.B. Accessories

There are three accessory items that are used with the Wizard® MagneSil® Sequencing Reaction Clean-Up System.

Plate Clamp 96 (Cat.# V8251)

The 8- and 96-tip liquid handling systems require a uniformly flat well trough for efficient solution transfer. However, multiwell PCR plates have some warpage directly from the manufacturer. This situation is often worsened by the thermal cycling process. Promega offers the Plate Clamp 96 to eliminate warpage in 96-well PCR plates and create a uniformly flat well trough for efficient liquid transfer.

The Plate Clamp 96 is designed to be used with a multiwell plate without a skirt (e.g., Robbins Scientific CyclePlate®-96ET, Cat.#1055-00-D or USA Scientific TempPlate II, Cat.# 1402-9600). The clamp consists of an upper and lower piece that fit on either side of a PCR plate. The assembly snaps together to hold the plate flat as shown in Figure 2, Panels A and B. The PCR plate can be inserted into the assembly either before or after thermal cycling.

Plate Stand (Cat.# V8261)

The Plate Stand (Figure 2, Panel C) has the footprint of a standard multiwell plate and is used to position the Plate Clamp 96 assembly in a specific location on the deck of a robotic workstation.

MagnaBot® II Magnetic Separation Device (Cat.# V8351)

The MagnaBot® II Device is designed specifically to work with a 96-well PCR plate. When a 96-well PCR plate containing MagneSil® Paramagnetic Particles is placed on the unit (Figure 2, Panel D), the particles are drawn to the same side and off the bottom of each well (Figure 1). This allows quantitative removal of liquids.

5. 384-Well Plate Procedure

We have developed a protocol for use of the Wizard® MagneSil® Sequencing Reaction Clean-Up System in 384-well plates using the Biomek® FX workstation (see **www.promega.com/tbs/ep010/ep010.html**). For more information about automated high-throughput purification of sequencing reactions in 384-well plates, go to: **www.promega.com/automethods/**

6. Composition of Buffers and Solutions

formamide/blue dextran/EDTA loading solution (use with ABI PRISM® 377 DNA Sequencer)

300µl Hi-Di™ formamide (Applied Biosystems, Cat.# 4311320)
60µl 25mM EDTA with 50mg/ml blue dextran (Applied Biosystems, Cat.# 402055)

90% ethanol wash solution

95ml 95% ethanol
5ml nuclease-free water

DNA sequencing 5X buffer

250mM Tris-HCl (pH 9.0 at 25°C)
100mM $MgCl_2$

7. 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
Low signal strength	May be due to inefficient capture of sequencing extension products. Increase the length of the binding step and/or increase the number of mixes performed during the binding step.
	Could be due to loss of particles throughout the procedure. Decrease the speed of aspiration, increase magnetization time and ensure that tips are offset from the magnetized particle pellet.
Signal gradient across plate	May be due to inefficient mixing of particles prior to binding. Increase speed and number of mixes. During mixing position tips to aspirate low, then dispense high in the well. Next, reverse the technique by aspirating high and dispensing low.
	Inefficient release of sequencing extension products may contribute to signal gradients on the plate. Ensure that the final ethanol wash is completely removed before proceeding to the elution step. Also, add a room-temperature incubation step to allow ethanol to evaporate.
"Dye blobs" present	May be due to inefficient removal of binding/wash solution. Ensure quantitative removal of solution after capture steps.
	Inefficient particle washing. Ensure that particles are resuspended completely during wash steps.

8. References

1. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**, 5463–67.
2. Prober, J.M. *et al.* (1987) A system for rapid DNA sequencing with fluorescent chain-terminating dideoxynucleotides. *Science* **238**, 336–41.
3. Rosenblum, B.B. *et al.* (1997) New dye-labeled terminators for improved DNA sequencing patterns. *Nucleic Acids Res.* **25**, 4500–4.

9. Related Products

Wizard® MagneSil® Plasmid Purification System

Product	Size	Cat.#
Wizard® MagneSil® Plasmid Purification System	4 × 96 preps	A1630
	8 × 96 preps	A1631
MagneSil® BLUE	100ml	A2201
MagneSil® RED	100ml	A1641
Cell Resuspension Solution	500ml	A7114
Cell Lysis Solution	500ml	A7124
Neutralization Solution	500ml	A7132

Accessories

Product	Cat.#
Plate Clamp 96	V8251
Plate Stand	V8261
MagnaBot® II Magnetic Separation Device	V8351

Wizard® SV 96 Plasmid DNA Purification System

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 96 Wash Solution	185ml	A1311
Wizard® SV 96 Binding Plates	10 pack	A2271
Wizard® SV 96 Lysate Clearing Plates	10 pack	A2241
Vac-Man® 96 Vacuum Manifold	96-well capacity	A2291
Collar for the Vac-Man® 96 Vacuum Manifold	1 each	A2311

[a]U.S. Pat. Nos. 6,027,945 and 6,368,800, Australian Pat. No. 732756, Mexican Pat. No. 209436 and other patents pending.

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Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

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.

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