TECHNICAL BULLETIN

Wizard MagneSil Tfx™ System

Instructions for Use of Product
A2380

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Wizard MagneSil Tfx™ System

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

1. Description

The Wizard MagneSil Tfx™ System[a,b,c] 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 transfection as well as for other standard molecular biology techniques. The use of MagneSil® Paramagnetic Particles for lysate clearing as well as DNA capture circumvents the need for centrifugation or vacuum manifolds, allowing DNA purification with the Wizard MagneSil Tfx™ System to be completely automated.

DNA purified with the Wizard MagneSil Tfx™ System is low in common plasmid prep impurities. Chemical contaminants as well as RNA, protein, and endotoxin are greatly reduced to provide high-quality plasmid DNA suitable for transfection. The amount of DNA used in transfections will vary depending on the transfection reagent and the cell line used and should be optimized whenever a new transfection reagent or cell line is examined.

The protocol herein has been developed for use on a Biomek® FX robotic workstation. The procedure requires no manual interventions and takes approximately 45 minutes to process a single 96-well plate. The protocol can be adapted to other robotic workstations. For information on an automated protocol for your system go to: **www.promega.com/automethods/** and provide your contact information. An Automation Support Team member will contact you regarding a protocol for use with your particular system.

The Wizard MagneSil Tfx™ System uses alkaline-SDS lysis to generate the bacterial lysate and incorporates MagneSil® Paramagnetic Particles for both lysate clearing and plasmid purification. The procedure is performed using a number of simple steps:

Alkaline-lyse the bacterial cell pellets

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Clear lysate using MagneSil® BLUE

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Remove endotoxin with Endotoxin Removal Resin

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Capture plasmid on MagneSil® RED

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Remove contaminants with 4/40 Wash Solution

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Wash with 80% ethanol

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Elute with Nuclease-Free Water

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Wizard MagneSil Tfx™ System	**4 × 96 preps**	**A2380**

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

- 150ml Cell Resuspension Solution (CRA)
- 150ml Cell Lysis Solution (CLA)
- 150ml Neutralization Solution (NSA)
- 38ml MagneSil® BLUE
- 19ml Endotoxin Removal Resin
- 30ml MagneSil® RED
- 38ml 4/40 Wash Solution
- 2 × 25ml Nuclease-Free Water

Storage Conditions: Store all Wizard MagneSil Tfx™ System components at room temperature (22–25°C). See the product label for expiration date.

3. System Requirements

This protocol requires a Biomek® FX robotic workstation with the following ALPs (all part numbers refer to Beckman products):

- Tip Loader ALP (Part# 719856)
- 96-well Tipwash ALP (Part# 719804)
- 3 × single-position labware ALP (Part# 719360)
- 16-position labware ALP (Part# 719360)
- circulating water bath that heats to 55–65°C
- single robotic arm fitted with a 96-well pipetting head
- heating/cooling ALP (Part# 719361)
- linear shaker ALP (Part# 719352)

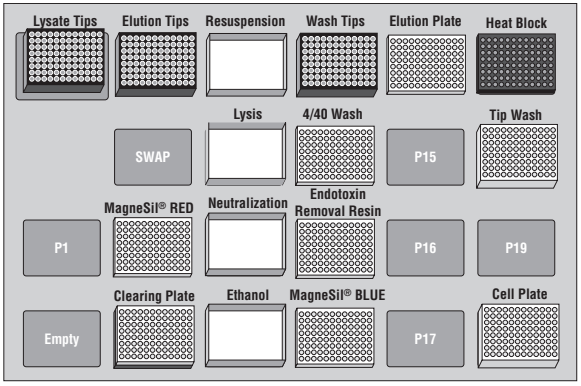

Figure 1. Diagram of the deck layout.

Materials to be Supplied by the User

(Solution compositions are provided in Section 7.A.)

In addition to the ALPs needed for the Biomek® FX robotic workstation, the following items are needed to perform plasmid DNA isolation with the workstation:

- 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)
- Heat Transfer Block (Cat.# Z3271)
- 4 × 96-well reservoirs, pyramid bottom (Innovative Microplate Cat.# S30014)
  Note: The Innovative Microplate reservoirs are ideal because their structure allows a low dead volume. If a different reservoir is used, larger dead volumes may necessitate re-using solutions.

To process one plate of 96 samples:

- deep-well (2ml) 96-well plate (e.g., Beckman deep-well multiwell plate, #140504)
  Note: If deep-well plates other than the Beckman plates are used, pipetting settings for the deep-well stations will have to be optimized.
- 3 boxes of 96 disposable P250 tips (Beckman Cat.# 717251)
- 6 × 96-well plates (Greiner Cat.# 650101 or Promega Cat.# A9161)
- 50ml of 80% ethanol
- 25ml of isopropanol

4. Protocols

4.A. Preparation of Cell Pellets

Bacterial cells should contain high-copy number-plasmids. For information on selecting and growing bacterial strains, see Sections7.B–C. As much as 6.0 $O.D._{600}$ of total cell mass may be processed per plate well.

1. Grow bacterial cells overnight at 37°C in up to 1ml of culture volume in a 2ml deep-well culture plate with square wells (e.g., Beckman deep-well titer plate, item# 140504).
2. Pellet cells by centrifuging for 15 minutes at 1,500 × *g* in a tabletop centrifuge. Pour off the supernatant and blot the plate upside down on a paper towel to remove excess liquid.
3. Samples can be processed immediately or covered and stored at −20°C for several weeks. If using a frozen plate, make sure the pellets warm to room temperature before beginning the protocol.

4.B. Robotic Setup

Follow these instructions to prepare plates and reservoirs for the robot deck.

1. Add 25ml of isopropanol to the 4/40 Wash Solution bottle and mix by shaking.
2. Add the following solutions to a 96-well Greiner plate, one plate per solution:
   - 1 plate with 50µl/well MagneSil® BLUE
   - 1 plate with 50µl/well MagneSil® RED

   Shake the MagneSil® BLUE and RED bottles until the resin is resuspended.
   - 1 plate with 25µl/well Endotoxin Removal Resin
   - 1 plate with 125µl/well 4/40 Wash Solution (“4/40 wash” plate, Figure 1)
   - 1 plate with 100µl/well Nuclease-Free Water (“Elution Plate”, Figure 1)

3. Add the following solutions directly to the reservoirs:
   - 25ml Cell Resuspension Solution (CRA)
   - 25ml Cell Lysis Solution (CLA)
   - 25ml Neutralization Solution (NSA)
   - 50ml of 80% ethanol
4. Set the heating ALP to 55–65°C.
5. Place the 96-well Greiner plates and reservoirs on the robot deck in the positions indicated in Figure 1 or as suggested in the electronic protocol under Setup.

4.C. Robotic Protocol

1. Add 90µl of Cell Resuspension Solution (CRA) to each plate well. Resuspend the bacterial pellets by pipetting and shaking.

   Note: Cell resuspension is very important. If the pellets are not completely resuspended, plasmid quality and yield can be affected. If biomass is too high, cell resuspension may be incomplete. During trial runs, stop the protocol after the resuspension step and check for resuspension. If resuspension is incomplete, increase shaking time.
2. Add 120µl of Cell Lysis Solution (CLA) to each well and mix by shaking.

   Complete lysis is important for yield and quality. Perform trial runs, checking for the completeness of lysis. If lysis is incomplete, decrease the amount of biomass.
3. Add 120µl of Neutralization Solution (NSA). Mix the lysate by pipetting and shaking.
4. Add 25µl MagneSil® BLUE. Mix the lysate by pipetting and shaking.
5. Transfer the lysate to a new 96-well Greiner plate and place the plate on the MagnaBot® 96. Allow the lysate to clear. A tight pellet should form in the corner of each well, which allows a pipette tip to descend to the center of the well without disturbing the pellet.
6. Transfer the cleared lysate to the plate containing the Endotoxin Removal Resin and mix by pipetting. The resin and lysate are mixed by pipetting and not by shaking because volumes are too large for shaking.
7. Transfer the plate containing the lysate and Endotoxin Removal Resin to the MagnaBot® 96 and allow the lysate to clear.

   Note: There should be no negative effects if a small amount of resin and debris is transferred to the endotoxin removal plate.
8. Transfer the cleared lysate to the plate containing the MagneSil® RED and mix by pipetting.
9. Transfer the plate containing the lysate and MagneSil® RED to the MagnaBot® 96 and allow the lysate to clear. Remove and discard the lysate.
10. Remove the plate containing MagneSil® RED from the magnet. Add 100µl of 4/40 Wash Solution and mix by pipetting.

11. Transfer the plate to the MagnaBot® 96. Allow the solution to clear, and then discard the 4/40 Wash Solution.
12. Remove the plate containing MagneSil® RED from the magnet. Add 100µl of 80% ethanol to each well. Mix by shaking.
13. Transfer the plate to the MagnaBot® 96. Allow the solution to clear, and then discard the 80% ethanol wash.
14. Repeat the ethanol step 2 times, for a total of 3 ethanol washes. It is important to remove as much ethanol as possible after the final wash.
15. Transfer the plate containing MagneSil® RED to the heating block for 7.5 minutes.

    Note: If a heating/cooling ALP is unavailable, the plate can be dried at room temperature for 10 minutes or longer.
16. Remove the plate from the heating block. Add 100µl of Nuclease-Free Water, and then mix by pipetting.

(!) Avoid contaminating the Nuclease-Free Water because biological contaminants will have serious negative effects on transfection.

17. Transfer the plate to the MagnaBot® 96 and allow the solution to clear. Remove the Nuclease-Free Water containing the plasmid and transfer it to a final elution plate. If small amounts of resin are carried over to the elution plate, remove them by placing the plate on the MagnaBot® 96 for 5 minutes and then transferring the eluate to a new plate.

5. 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
Incomplete resuspension of cells (cell pellet still visible)	Cells stored too long at –20°C. Cells should only be stored 2–4 weeks, maximum, at –20°C. Shake an additional 5 minutes.
	Too many cells, 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 not thawed completely. When using frozen cells, ensure that they are completely thawed. Allow the cells to sit at room temperature at least 15 minutes.
Turbidity observed in cleared lysate	Too long at the neutralization step. **Do not** shake longer than 4 minutes. The lysate will remain cloudy and decreased plasmid DNA yield will result.
	Incomplete lysis. Make sure frozen cells are completely thawed. Increase lysis time from 3 to 5 minutes.

Symptoms	Causes and Comments
Turbidity observed in cleared lysate (continued)	Too many cells, 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.
Compact pellet does not form at magnet corner, and complete removal of cleared lysate is not possible	Too many cells, biomass greater than 6.0 O.D.$_{600}$. Do not attempt to process more than 6.0 O.D.$_{600}$ per well.
	Growth medium is interfering with protocol. We recommend Terrific broth or CIRCLEGROW® medium for this protocol.
	Magnetic flux may be insufficient. Use only the Greiner 96-well plates or plates of the same design.
Downstream applications are problematic	Insufficient washing. Thorough washing is required to remove salts that might interfere with downstream applications. Ensure that the magnetic particles are thoroughly suspended in the well by the action of the shaker or by pipetting at each wash step.
	Alcohol carryover. Ensure that all the wash solution is removed from the well. If alcohol is spilled while shaking, reduce the amplitude.
Low DNA yield	Incomplete resuspension. Thoroughly resuspend the particles after adding the Nuclease-Free Water.
	Insufficient mixing of binding resin and lysate. The binding resin and lysate must be thoroughly mixed to ensure maximum binding.
Particle carryover to final elution plate	Aspiration rate too high. Reduce the aspiration rate.
	Particles adhering to tip walls. If re-using tips or using fixed tips, rinse them to remove adherent particles.
	Add a second magnetic step to remove particles adhering to tip walls.

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6. Related Products

Product	Size	Cat.#
PureYield™ Plasmid Midiprep System	25 preps	A2492
	100 preps	A2495
	300 preps	A2496
PureYield™ Plasmid Maxiprep System	10 preps	A2392
	25 preps	A2393
Wizard® MagneSil® Plasmid Purification System	4 × 96 preps	A1630
	8 × 96 preps	A1631
	100 × 96 preps	A1635
Wizard® SV 96 Plasmid Purification Systems	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

Product	Size	Cat.#
MagnaBot® 96 Magnetic Separation Device	1 each	V8151
Vac-Man® 96 Vacuum Manifold	96-well capacity	A2291
Endotoxin Removal Resin	100ml	A2191
4/40 Wash Solution	115ml	A2221

7. Appendix

7.A. Composition of Buffers and Solutions

Cell Resuspension Solution (CRA)

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

Cell Lysis Solution (CLA)

0.2M NaOH
1.0% SDS

Neutralization Solution (NSA)

1.32M potassium acetate (pH 4.8)

4/40 Wash Solution

7M guanidine hydrochloride

Add isopropanol as instructed on the bottle label. The final concentration of guanidine-HCl is 4.2M.

80% ethanol wash solution

Prepare 100ml of 80% ethanol wash solution by adding 80ml of 100% ethanol to 20ml of high-quality water (or 84ml of 95% ethanol to 16ml of high-quality water). Store at 20–25°C. Prepare 33ml/reservoir per 96-well plate processed.

7.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 *endA*1 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 1 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	Select96™	TOP10
EndA$^+$ Strains:			
BL21(DE3)	CJ236	HB101	JM83
JM101	JM110	LE392	MC1061
NM522*	NM55	4P2392	PR700*
Q358	RR1TB1	TG1	Y1088*
BMH 71-18	ES1301		

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

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7.C. Preparation of *E. coli* Cultures for Plasmid Isolation

Dispense 0.5–1.5ml 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® or Terrific Broth 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 $O.D._{600}$ 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.D. $O.D._{600}$ Calculation

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

[a]U.S. Pat. Nos. 6,284,470 and 7,078,224, Japanese Pat. No. 4551568 and other patents pending.

[b]U.S. Pat. No. 6,194,562 and other patents pending.

[c]U.S. Pat. Nos. 6,027,945, 6,368,800 and 6,673,631 and other patents.