Promega

Technical Bulletin

Wizard® DNA Clean-Up System

INSTRUCTIONS FOR USE OF PRODUCT A7280.

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PRINTED IN USA.
Revised 4/06

Part# TB141

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Wizard® DNA Clean-Up System

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I. Description..........1

II. Product Components and Storage Conditions..........2

III. Protocol for DNA Purification Using a Vacuum Manifold..........2

IV. Protocol for DNA Purification Without a Vacuum Manifold..........3

I. Description

Concentration of DNA samples, removal of small nucleotides from reactions and purification of DNA from proteins or salts all require organic extractions and precipitation, which can result in low and variable recoveries. The concern over recovery increases when working with amounts of DNA in the 50–500ng range.

The Wizard® DNA Clean-Up System[a] provides a simple and effective way to purify linear and circular DNA (200–50,000bp) from the following:

- restriction enzymes, including heat-stable restriction enzymes
- phosphatases and kinases
- DNA polymerases, including *Taq* DNA Polymerase
- exonucleases and endonucleases, including DNase I but not RNases
- mononucleotides
- salts

The entire procedure can be completed in 15 minutes or less, with no organic extractions or ethanol precipitation. DNA is eluted from the Wizard® Clean-Up Resin in water or TE buffer, ready for use.

For convenience and efficiency, multiple samples may be processed at one time with the Vac-Man® (Cat.# A7231) or Vac-Man® Jr. (Cat.# A7660) Laboratory Vacuum Manifold.

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II. Product Components and Storage Conditions

Product	Size	Cat.#
Wizard® DNA Clean-Up System	100 preps	A7280

For Laboratory Use. Each system contains sufficient reagents for 100 samples. Includes:

- 100ml Wizard® DNA Clean-Up Resin
- 100 Wizard® Minicolumns
- 100 Syringe Barrels (3cc)
- 1 Protocol

Storage Conditions: Store at room temperature (22–25°C). Protect the resin from exposure to direct sunlight.

III. Protocol for DNA Purification Using a Vacuum Manifold

Multiple samples can be easily processed simultaneously using the Vac-Man® Laboratory Vacuum Manifold. The Wizard® DNA Clean-Up System is not suitable for use with RNA because percent recoveries are less than 50%.

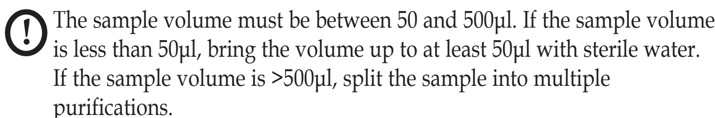
Notes:

1. Thoroughly mix the Wizard® DNA Clean-Up Resin before removing an aliquot. If crystals or aggregates are present, dissolve by warming the resin to 37°C for 10 minutes. The resin itself is insoluble. Cool to 25–30°C before use.

2. The binding capacity of 1ml of resin is approximately 20μg of DNA.

Materials to Be Supplied by the User

- 80% isopropanol (2-propanol, reagent grade)
- prewarmed (65–70°C) deionized water or TE buffer



The sample volume must be between 50 and 500μl. If the sample volume is less than 50μl, bring the volume up to at least 50μl with sterile water. If the sample volume is >500μl, split the sample into multiple purifications.

1. Use one Wizard® Minicolumn for each sample.

2. Attach the provided Syringe Barrel to the Luer-Lok® extension of each Minicolumn. Insert the tip of the Minicolumn/Syringe Barrel assembly into the vacuum manifold.

Mix the resin before use.

3. Add 1ml of Wizard® DNA Clean-Up Resin to a 1.5ml microcentrifuge tube. Add the sample (50–500μl) to the Clean-Up Resin and mix by inverting several times.

III. Protocol for DNA Purification Using a Vacuum Manifold (continued)

4. Pipet the resin/DNA mix into the Syringe Barrel. Apply a vacuum to draw the solution through the Minicolumn. Break the vacuum to the Minicolumn.
5. To wash the column, add 2ml of 80% isopropanol to the Syringe Barrel, and re-apply a vacuum to draw the solution through the Minicolumn.
6. 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 maximum speed (10,000 x *g*) in a microcentrifuge for 2 minutes to remove any residual isopropanol.
7. Transfer the Minicolumn to a new microcentrifuge tube. Apply 50µl (see Table 1) of prewarmed (65–70°C) water or TE buffer (10mM Tris-HCl [pH 7.6], 1mM EDTA) to the Minicolumn and wait 1 minute. (The DNA will remain intact on the Minicolumn for up to 30 minutes.) Centrifuge the Minicolumn for 20 seconds at maximum speed (10,000 x *g*) to elute the bound DNA.

   ! **Elute** DNA >20kb with water or TE buffer prewarmed to 80°C.
8. Remove and discard the Minicolumn. The purified DNA may be stored in the microcentrifuge tube at 4°C or –20°C.

IV. Protocol for DNA Purification Without a Vacuum Manifold

It is possible to purify the DNA using a 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.

One disposable 3ml Luer-Lok® syringe is required for each clean-up (e.g., Becton, Dickinson and Company, Cat.# 309585).

Notes:

1. Thoroughly mix the Wizard® DNA Clean-Up Resin before removing an aliquot. If crystals or aggregates are present, dissolve by warming the resin to 37°C for 10 minutes. The resin itself is insoluble. Cool to 25–30°C before use.
2. The binding capacity of 1ml of resin is approximately 20µg of DNA.

IV. Protocol for DNA Purification Without a Vacuum Manifold (continued)

Materials to Be Supplied by the User

- 80% isopropanol (2-propanol, reagent grade)
- prewarmed (65–70°C) deionized water or TE buffer
- disposable 3ml Luer-Lok® syringes

(!) The sample volume must be between 50 and 500µl. If the sample volume is less than 50µl, bring the volume up to at least 50µl with sterile water. If the sample volume is >500µl, split the sample into multiple purifications.

1. Use one Wizard® Minicolumn for each sample. Remove and set aside the plunger from a 3ml disposable syringe. Attach the Syringe Barrel to the Luer-Lok® extension of each Minicolumn.

(!) **Mix** the resin before use.

2. Add 1ml of Wizard® DNA Clean-Up Resin to a 1.5ml microcentrifuge tube. Add the sample (50–500µl) to the Clean-Up Resin and mix by gently inverting several times.

3. Pipet the Wizard® DNA Clean-Up Resin containing the bound DNA into the Syringe Barrel. Insert the syringe plunger slowly and gently push the slurry into the Minicolumn with the syringe plunger.

4. Detach the syringe from the Minicolumn and remove the plunger from the syringe. Reattach the Syringe Barrel to the Minicolumn. To wash the column, pipet 2ml of 80% isopropanol into the syringe. Insert the plunger into the syringe and gently push the solution through the Minicolumn.

5. Remove the Syringe Barrel and transfer the Minicolumn to a 1.5ml microcentrifuge tube. Centrifuge the Minicolumn for 2 minutes at maximum speed (10,000 x *g*) to dry the resin.

6. Transfer the Minicolumn to a new microcentrifuge tube. Apply 50µl (see Table 1) of prewarmed (65–70°C) water or TE buffer (10mM Tris-HCl [pH 7.6], 1mM EDTA) to the Minicolumn and wait 1 minute. (The DNA will remain intact on the Minicolumn for up to 30 minutes.) Centrifuge the Minicolumn for 20 seconds at maximum speed (10,000 x *g*) to elute the bound DNA fragment.

(!) **Elute** DNA >20kb with water or TE buffer prewarmed to 80°C.

7. Remove and discard the Minicolumn. The purified DNA may be stored in the microcentrifuge tube at 4°C or –20°C.

Table 1. Elution Volume vs. Percent Recovery of DNA (3kb Plasmid).

Elution Volume	Approximate % Recovery
50µl	100%
40µl	100%
30µl	100%
20µl	75%
10µl	55%

Table 2. Various Amounts of Double-Stranded DNA (3kb Plasmid) vs. Percent Recovery.

Amount of Plasmid DNA	Approximate % Recovery
50ng	≥80%
500ng	≥90%
1µg	≥90%
5µg	≥90%
10µg	76%
20µg	65%
40µg	40%

Table 3. Various Amounts of Single-Stranded DNA (M13mp18) vs. Percent Recovery.

Amount of ssDNA	Approximate % Recovery
50ng	≥50%
500ng	≥50%
1µg	≥60%
5µg	≥60%

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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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