TECHNICAL BULLETIN

Wizard® SV 96 Genomic DNA Purification System

Instructions for Use of Products
A2370 and A2371

Revised 4/15
TB303

Wizard® SV 96 Genomic DNA Purification 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® SV 96 Genomic DNA Purification System provides a high-throughput 96-well format for fast, simple preparation of intact, purified genomic DNA from different sample types, including mouse tail clippings, animal tissue and tissue culture cells. The technique allows automation on liquid-handling workstations. Purification of genomic DNA from 96 samples takes less than one hour and is achieved without centrifugation or precipitation. The isolated genomic DNA is high-quality and serves as an excellent template for agarose gel analysis, restriction enzyme digestion and PCR. Typical yields from various tissue sources are shown in Table 1.

The Wizard® SV 96 Genomic DNA Purification System requires the use of the Vac-Man® 96 Vacuum Manifold or similar 96-well manifold for manual DNA purification. For genomic DNA purification on automated liquid-handling workstations, vacuum manifolds specific to the workstation may be required. Genomic DNA is purified from lysates using 96-well vacuum filtration. Washing the bound DNA does not require disassembly of the manifold, and filtrate waste products are delivered directly to a vacuum trap, eliminating the need to empty waste collection vessels during DNA purification. Genomic DNA is collected by elution into a 96-Well Deep Well Plate.

1. Description (continued)

Table 1. Genomic DNA Yield From Various Tissues.

Sample	Amount	Average Yield
Tail Clipping	20mg	20µg
Liver	20mg	15µg
Heart	20mg	10µg
Brain	20mg	6µg
CHO cells	1×10^6 cells	5µg
NIH3T3 cells	1×10^6 cells	9µg
293 cells	1×10^6 cells	8µg

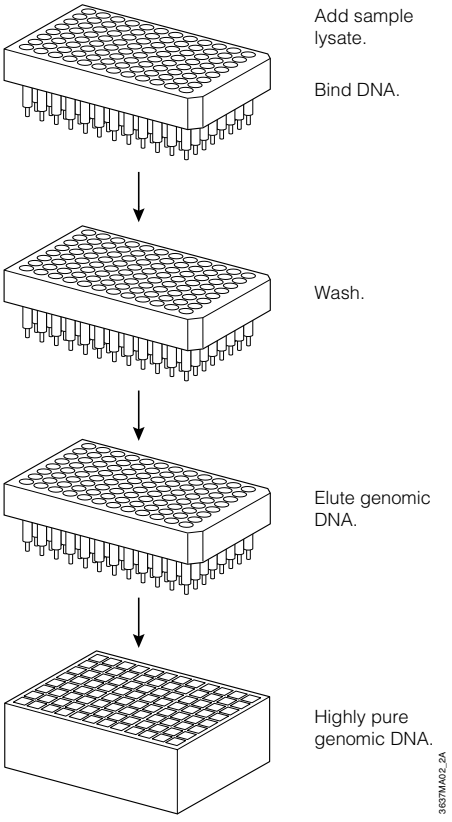

Figure 1. Genomic DNA isolation and purification using the Wizard® SV 96 Genomic DNA Purification System.

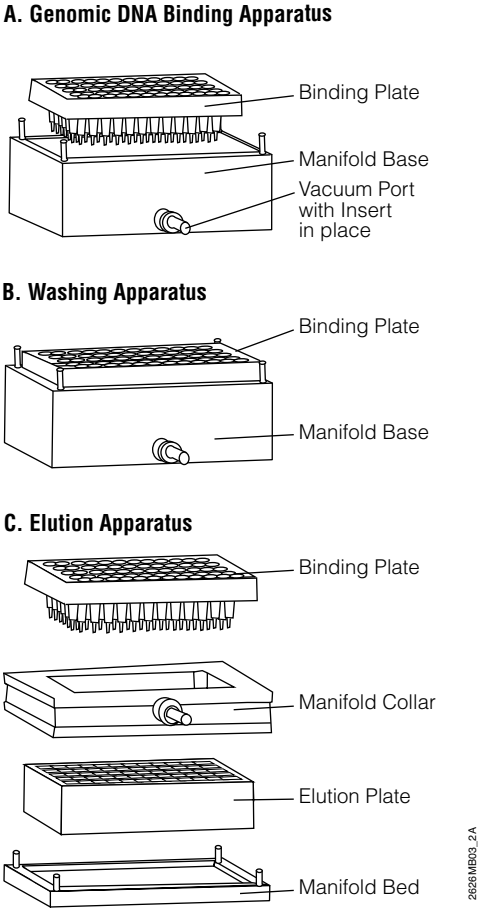

Figure 2. The Vac-Man® 96 Vacuum Manifold (Cat.# A2291) with the Wizard® SV 96 Genomic DNA Purification System. Panels A, B and C show the manifold and plate combinations necessary to accomplish genomic DNA binding, washing and elution, respectively, for manual genomic DNA purification. For genomic DNA purification on automated liquid handlers, vacuum manifold equipment specific for the workstation may be required.

Please note that an appropriate liquid trap should be placed between the Vacuum Manifold and your vacuum pump. An instructional video on how to set up a Vac-Man® 96 Vacuum Manifold is available at: **www.promega.com/vacman96/**

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Wizard® SV 96 Genomic DNA Purification System	**1 × 96 preps**	**A2370**

Includes:

- 1 Binding Plate
- 1 96-Well Deep Well Plate
- 50ml Nuclei Lysis Solution
- 30ml 0.5M EDTA (pH 8.0)
- 50ml Wizard® SV Lysis Buffer
- 185ml Column Wash Solution (CWA; concentrated)
- 1ml RNase A Solution, 4mg/ml
- 150ml Nuclease-Free Water

PRODUCT	SIZE	CAT.#
Wizard® SV 96 Genomic DNA Purification System	**4 × 96 preps**	**A2371**

Includes:

- 4 Binding Plates
- 4 96-Well Deep Well Plates
- 2 × 50ml Nuclei Lysis Solution
- 30ml 0.5M EDTA (pH 8.0)
- 3 × 50ml Wizard® SV Lysis Buffer
- 2 × 370ml Column Wash Solution (CWA; concentrated)
- 3 × 1ml RNase A Solution, 4mg/ml
- 2 ×150ml Nuclease-Free Water

Storage Conditions: Store all Wizard® SV 96 Genomic DNA Purification System components at 22–25°C. See system label for expiration date.

Note: If purifying genomic DNA from tissue samples, such as mouse tails, **proteinase K must be purchased separately**. For one plate (96 samples), 40mg of proteinase K will be required (Cat.# V3021, 100mg).

3. Isolation of Genomic DNA from Mouse Tail Clippings and Animal Tissues

Materials to Be Supplied by the User

- Proteinase K (20mg/ml solution in nuclease-free water) (Cat.# V3021 or Sigma Cat.# P2308). Proteinase K must be qualified nuclease-free.
- Vac-Man® 96 Vacuum Manifold (Cat.# A2291)
- 55°C water bath
- vacuum trap for waste collection (e.g., Fisher Cat.# 10-182-50B, 1L size)
- vacuum pump capable of 15–20 inches of Hg (e.g., Fisher Cat.# 01-092-29)
- vacuum tubing
- single or multichannel pipettors capable of dispensing 10–1,000µl
- adhesive plate sealers (foil)
- 96-well deep well plate for proteinase K digestion

3.A. Preparation of Solutions

Prepare the following solutions prior to beginning the Wizard® SV 96 Genomic DNA Purification System protocol:

Proteinase K Solution: Resuspend proteinase K with Nuclease-Free Water to a concentration of 20mg/ml working solution. Dispense the proteinase K into working volumes determined by the average number of preps done at a time. Store proteinase K solution at –20°C and thaw on ice. Avoid multiple freeze-thaw cycles of the proteinase K solution, as this will result in decreased activity.

Column Wash Solution (CWA): Add 95% ethanol to the Column Wash Solution (CWA) bottle as directed on the bottle label. Label the bottle to indicate that ethanol has been added. Seal well and store at room temperature.

Digestion Solution Master Mix: For every tissue sample, combine the following reagents in a tube and store on ice until use:

Digestion Solution Master Mix	Volume per Sample	Total Volume for 96 Samples
Nuclei Lysis Solution	200µl	22.0ml
0.5M EDTA (pH 8.0)	50µl	5.5ml
proteinase K, 20mg/ml	20µl	2.2ml
RNase A Solution, 4mg/ml	5µl	550µl
Total Volume	**275µl**	**30.25ml**

3.B. Preparation of Mouse Tail and Tissue Lysates

1. Use a 0.5–1.2cm mouse tail clipping from the tip of the tail or up to 20mg of other tissue sample. A 1.2cm mouse tail clipping usually weighs approximately 20mg. Cut the 20mg mouse tail clipping or tissue sample into two equally sized pieces and place the pieces into a 96-well, deep well plate (provided by the user).

   Note: Tissue mass cannot exceed the recommended amount, or the column will clog. Mouse tail clippings must be from the terminal 2cm of the mouse tail. Samples further from the tip of the tail will contain excess cartilagenous material that will clog the minicolumn.

2. Add 275µl of the prepared Digestion Solution Master Mix to each sample in the 96-well deep well plate. If the mouse tail clipping or tissue sample is not covered by the Digestion Solution Master Mix, cut the tissue into smaller pieces. Be sure that the sample is completely covered with Digestion Solution Master Mix. Cover the plate with an adhesive seal (provided by the user).

3. Place the plate in a 55°C water bath and incubate overnight (16–18 hours). Be sure that the water in the incubator does not cover the sample plate. It is not necessary to shake the plate during the incubation.

   Optional: If there is undigested hair and cartilage after the overnight proteinase K digestion, spin the plate in a centrifuge at 2,000 × *g* to pellet any undigested sample. Transfer the supernatant to a new 96-well, deep well plate (provided by the user). Proceed with addition of the Wizard® SV Lysis Solution.

3.C. Purification of Genomic DNA from Prepared Tissue Lysates

1. Following overnight incubation at 55°C, remove the seal and dispense 250µl of the Wizard® SV Lysis Buffer into each well of the deep-well plate containing lysate. **Note:** Lysate must be warm during processing.

2. Mix the contents of each well by pipetting several times. Process the lysates as soon as possible after the Wizard® SV Lysis Buffer has been added (lysate must still be warm). If lysates cannot be processed immediately, the sample lysate plate may be frozen at –70°C. However, the lysates need to be warmed to 55°C for one hour before they are processed.

3. Prepare the Vacuum Manifold as shown in Figure 2. Place the Binding Plate in the Vacuum Manifold Base. Orient the Binding Plate in the Vacuum Manifold with the numerical column headers toward the vacuum port. Attach the vacuum line to the vacuum port on the Manifold Base.

4. Transfer the tissue lysates to the wells of the Binding Plate. Apply vacuum until all of the lysate has passed through the Binding Plate.

   Note: Vacuum pressure should be >15 inches of mercury for efficient processing. Some wells of the Binding Plate may empty more quickly than other wells. To maintain strong vacuum through the wells, cover the empty wells to reduce vacuum leak.

5. Verify that ethanol has been added to the Column Wash Solution (CWA).

6. Add 1ml of Column Wash Solution (CWA) to each well of the Binding Plate.

7. Apply vacuum until the Column Wash Solution (CWA) passes through the Binding Plate.

8. Repeat Steps 6 and 7 two more times for a total of 3 washes with the Column Wash Solution (CWA).

9. After the wells have emptied, continue to apply vacuum for an additional 6 minutes to allow the binding matrix to dry.

10. Turn off the vacuum. Release the vacuum line from the Manifold Base, and snap it into the vacuum port in the Vacuum Manifold Collar. Remove the Binding Plate from the Manifold Base. Blot by gently tapping onto a clean paper towel to remove residual ethanol; repeat if necessary to remove all residual ethanol.

11. Place the 96-Well Deep Well Plate in the Manifold Bed, and position the Vacuum Manifold Collar on top. Orient the plate with the numerical column headers toward the vacuum port.

12. Position the Binding Plate on top of the Manifold Collar. Place the Manifold Collar containing the Binding Plate on top of the 96-Well Deep Well Plate sitting on the manifold bed as shown in Figure 2. The Binding Plate tips must be centered on the Deep Well Plate wells, and both plates must be in the same orientation.

13. Add 250µl of room-temperature Nuclease-Free Water to each well of the Binding Plate and incubate for 2 minutes at room temperature.

    Optional: To improve DNA yield, heat the water to 65°C before adding it to the column for elution.

14. Apply vacuum until the Nuclease-Free Water passes through the Binding Plate.

15. Repeat Steps 13 and 14 for a total elution volume of 500µl.

    Note: Elution volumes of 500µl are recommended for optimal DNA yield from tissue samples. Elution volumes less than 500µl will concentrate the DNA but will decrease the total DNA yield.

16. Release the vacuum, and remove the Wizard® SV 96 Binding Plate. Carefully remove the Manifold Collar, making sure that the Deep Well Plate remains positioned in the Manifold Bed. If droplets are present on the top of the wells of the plate, gently tap the plate on the bench top until the droplets fall to the bottom of the plate. Eluate volumes may vary but are generally 440–450µl. Samples can be stored at –20°C or –70°C by covering the plate tightly with a plate sealer.

4. Isolation of Genomic DNA From Tissue Culture Cells

Materials To Be Supplied By The User

- 1X phosphate-buffered saline (PBS), sterile (for cultured cells)

4.A. Preparation of Solutions

Prepare the following solution prior to beginning the Wizard® SV 96 Genomic DNA Purification System protocol for tissue culture cells:

Column Wash Solution (CWA): Add 95% ethanol to the Column Wash Solution (CWA) bottle as directed on the bottle label. Label the bottle to indicate that ethanol has been added. Seal well and store at room temperature.

4.B. Purification of Genomic DNA from Tissue Culture Cells

Use the following protocol for lysis of cultured cells grown in a 96-well tissue culture plate. Use at least 1×10^4 cells to a maximum of 5×10^6 cells per purification. The number of cells may need to be adjusted depending on cell type and function.

1. Wash the cells once with sterile 1X PBS.
2. Add 150µl of Wizard® SV Lysis Buffer to the washed cells. Mix by pipetting.
3. Freeze cell lysates at –70°C if they are not going to be processed immediately.
4. Prepare the vacuum manifold as shown in Figure 2. Place the Binding Plate in the vacuum manifold base. Orient the Binding Plate in the vacuum manifold with the numerical column headers toward the vacuum port. Attach the vacuum line to the vacuum port on the Manifold Base.
5. Transfer the cell lysates to the wells of the Binding Plate. Apply vacuum until the lysates have all passed through the Binding Plate.
6. Verify that ethanol has been added to the Column Wash Solution (CWA). Add 1ml of Wash Solution to each well of the Binding Plate. Apply vacuum until the Wash Solution passes through the Binding Plate.
7. Repeat Step 6 two more times for a total of 3 washes with the Column Wash Solution (CWA).
8. After the wells have emptied, continue to apply vacuum for an additional 6 minutes to dry the binding matrix.
9. Turn off the vacuum. Release the vacuum line from the Manifold Base, and snap it into the vacuum port in the Vacuum Manifold Collar. Remove the Binding Plate from the Manifold Base. Blot by gently tapping onto a clean paper towel to remove residual ethanol; repeat if necessary to remove all residual ethanol.

   Note: RNA may be copurified with genomic DNA. To remove copurified RNA, add 2µl of RNase A Solution per 250µl of Nuclease-Free Water prior to elution of DNA from the column. Once eluted, incubate purified DNA at room temperature for 10 minutes. Alternatively, add the RNase A Solution (2µl) following elution from the Wizard® SV 96 Binding Plate.
10. Place the 96-Well Deep Well Plate in the Manifold Bed, and position the Vacuum Manifold Collar on top. Orient the plate with the numerical column headers toward the vacuum port.

11. Position the Binding Plate on top of the Manifold Collar. Place the manifold collar containing the Binding Plate on top of the Deep Well Plate sitting on the manifold bed as shown in Figure 2. The Binding Plate tips must be centered on the deep well elution plate and both plates must be in the same orientation.

12. Add 250µl of room temperature Nuclease-Free Water to each well of the Binding Plate and incubate for 2 minutes at room temperature.

    Note: An elution volume of 250µl is recommended for optimal yield from tissue culture cell samples. Elution in volumes less than 250µl will concentrate the DNA but will decrease the total DNA yield. Elution volumes greater than 250µl will not significantly improve yield.

    Optional: To improve DNA yield, heat the water to 65°C before adding it to the column for elution.

13. Apply a vacuum for 1 minute.

14. Release the vacuum, and remove the Binding Plate. Carefully remove the manifold collar, making sure that the Deep Well Plate remains positioned in the Manifold Bed. If droplets are present on the top of the wells of the plate, gently tap the plate on the bench top until the droplets fall to the bottom of the plate. Eluate volumes may vary but are generally 225µl. Samples can be stored at –20°C or –70°C by covering the plate tightly with a plate sealer.

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
Low A_{260} (low DNA yield)	Tissue lysate stored at –20°C or –70°C. Lysate that has been frozen may have a decreased amount of genomic DNA. For optimal performance, purify the DNA as soon as the lysate is prepared.
	Tissues have undergone multiple freeze-thaw cycles. Samples that have been frozen and thawed repeatedly may eventually experience DNA degradation. Use fresh tissue samples whenever possible.
	Tissue culture cells are low in genomic DNA. Yield of genomic DNA may vary depending on the number of cells used for the isolation. If genomic DNA yields are low, increase the amount of starting material processed to a maximum of 5×10^6 tissue culture cells.
	Wizard® SV Lysis Buffer not added to the tissue lysates or washed cells. Make sure the Wizard® SV Lysis Buffer is added to all samples.
	Sample lysates have been frozen but not thawed and warmed to 55°C. Process tissue lysates as soon as they are removed from 55°C incubation. If samples have cooled, place the lysates back at 55°C for 60 minutes and continue the purification.
	Steps not followed correctly or wrong reagents used. The Wizard® SV 96 Genomic DNA Purification System is a multistep process that requires that the correct reagents are used in the correct order. This ensures that the DNA remains bound to the membrane during purification. The Wizard® *Plus* SV DNA Purification System buffers are not compatible with this system and should not be used.
	Ethanol not added to the Column Wash Solution (CWA). Prepare the solutions as instructed in Sections 3.A and 4.A before beginning the procedure.

Symptoms	Causes and Comments
RNA contamination	RNase A was not added to the tissue lysate digestion solution. Add 2µl of RNase A solution to the final eluate and incubate at room temperature for at least 10 minutes.
	RNA was copurified with genomic DNA from tissue culture cells. Add 2µl of RNase A Solution to final eluate and incubate at room temperature for at least 10 minutes.
Clogged Wizard® SV 96 Binding Plate	Lysate too concentrated or too viscous to pipet easily. If the lysate is too viscous, dilute with Wizard® SV Lysis Buffer until it becomes easy to pipet. Then apply the entire lysate to a well of the Wizard® SV 96 Binding Plate.
	Too much tissue sample used in the lysate preparation. A **maximum of 20mg** of mouse tail or animal tissue can be used for lysate preparation.
	Too many cells processed. A maximum of 5×10^6 cells can be processed per column of the Wizard® SV 96 Binding Plate.
	Tissue lysate becomes too viscous when allowed to cool. Process tissue lysates as soon as they are removed from 55°C incubation. If samples have cooled, place the lysates back in the 55°C incubator for 60 minutes and continue with the purification.
	Proteinase K-treated mouse tail clippings contain a lot of undigested hair or cartilage. Mouse tail clippings must be collected from within the terminal 2cm of the tail tip. Clippings collected further from the tip will contain cartilaginous tissue that will clog the column.
	Mouse tail hair and cartilage are not easily digested by proteinase K. After proteinase K digestion centrifuge the sample at $2{,}000 \times g$ to pellet undigested sample. Transfer the supernatant to a new 96-well, deep well plate and proceed with the protocol.

5. Troubleshooting (continued)

Symptoms	Causes and Comments
Incomplete digestion of tissue samples	Make sure Proteinase K was added to the Digestion Solution Master Mix.
	Too much tissue sample used in the lysate preparation. A **maximum of 20mg** of mouse tail or animal tissue can be used for lysate preparation.
	Multiple freeze-thaw cycles of proteinase K.Multiple freeze-thaw cycles reduce the activity of proteinase K. Begin with a new proteinase K solution.
Vacuum steps are slow	Lysate too concentrated. If the lysate is difficult to pipette easily, the vacuum step to pass lysate through the Wizard® SV 96 Binding Plate may be slow.
	Vacuum pressure insufficient. A vacuum pressure >15 inches of mercury is required to use the Wizard® SV 96 Binding Plate.

6. Related Products

Please visit our online catalog at **www.promega.com** for a complete listing of our newest DNA Purification and Amplification products.

Product	Size	Cat.#
Wizard® SV Genomic DNA Purification System	50 preps	A2360
	250 preps	A2361
Wizard® Genomic DNA Purification Kit	100 isolations × 300µl	A1120
	500 isolations × 300µl	A1125
	100 isolations × 10ml	A1620
Proteinase K	100mg	V3021
PCR Master Mix	10 reactions	M7501
	100 reactions	M7502
	1,000 reactions	M7505
PCR Nucleotide Mix	200µl	C1141
	1,000µl	C1145

7. Summary of Changes

The following changes were made to the 4/15 revision of this document:

1. Added a note containing a link to an instructional video for setting up the vacuum manifold (Section 1 following Figure 2).
2. The document design was updated.