Technical Bulletin

Wizard® SV Genomic DNA Purification System

INSTRUCTIONS FOR USE OF PRODUCTS A2360, A2361 AND A2365.

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Wizard® SV Genomic DNA Purification System

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1. Description

The Wizard® SV Genomic DNA Purification System provides a fast, simple technique for the preparation of purified and intact DNA from mouse tails, tissues and cultured cells in as little as 20 minutes, depending on the number of samples processed. For this system, either a spin or vacuum ("SV") purification protocol can be used (Figure 1). Up to 20mg of tissue (mouse tail or animal tissue) or between 1×10^4 and 5×10^6 tissue culture cells can be processed per purification. The genomic DNA isolated with this system is of high quality and serves as an excellent template for agarose gel analysis, restriction enzyme digestion and PCR analysis. Table 1 provides typical yields of genomic DNA purified from a variety of sources.

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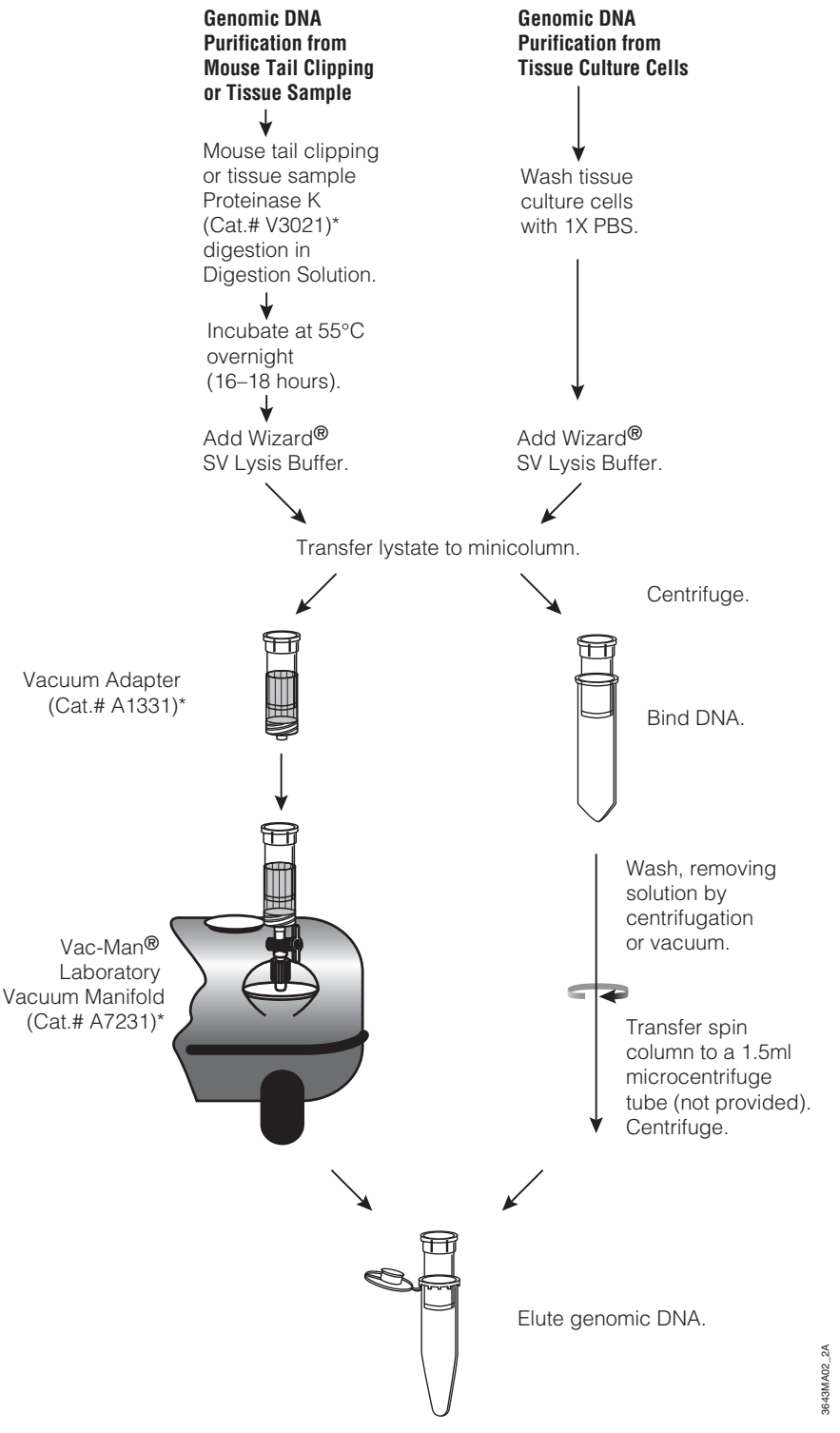

Figure 1. Overview of the Wizard® SV Genomic DNA Purification spin and vacuum protocols. *May be purchased separately.

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

2. Product Components and Storage Conditions

Product	Size	Cat.#
Wizard® SV Genomic DNA Purification System	10 preps	A2365

Includes:

- 1 Wizard® SV Minicolumns (pack of 10)
- 1 Collection Tubes (pack of 10)
- 5ml Nuclei Lysis Solution
- 1ml 0.5M EDTA (pH 8.0)
- 5ml Wizard® SV Lysis Buffer
- 50ml Column Wash Solution (CWA; concentrated)
- 250µl RNase A Solution
- 13ml Nuclease-Free Water

Product	Size	Cat.#
Wizard® SV Genomic DNA Purification System	50 preps	A2360

Includes:

- 1 Wizard® SV Minicolumns (pack of 50)
- 1 Collection Tubes (pack of 50)
- 50ml Nuclei Lysis Solution
- 30ml 0.5M EDTA (pH 8.0)
- 50ml Wizard® SV Lysis Buffer
- 185ml Column Wash Solution (CWA; concentrated)
- 250µl RNase A Solution
- 2 × 25ml Nuclease-Free Water

2. Product Components and Storage Conditions (continued)

Product	Size	Cat.#
Wizard® SV Genomic DNA Purification System	250 preps	A2361

Includes:

- 1 Wizard® SV Minicolumns (pack of 250)
- 1 Collection Tubes (pack of 250)
- 50ml Nuclei Lysis Solution
- 30ml 0.5M EDTA (pH 8.0)
- 2 × 50ml Wizard® SV Lysis Buffer
- 2 × 185ml Column Wash Solution (CWA; concentrated)
- 2 × 1ml RNase A Solution
- 150ml Nuclease-Free Water

Storage Conditions: Store all Wizard® SV 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 a total of 10 preps, 4mg of proteinase K is required, for 50 preps, 20mg of proteinase K is required and for 250 preps, 100mg of proteinase K is required.

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

Materials to Be Supplied by the User

- Proteinase K (20mg/ml solution in nuclease-free water) (Cat.# V3021 or Sigma Cat.# P2308). **Note:** Proteinase K must be qualified as nuclease-free.
- 55°C heat block or water bath
- 1.5ml microcentrifuge tubes
- microcentrifuge capable of 13,000 × *g* **or** Vac-Man® Laboratory Vacuum Manifold (Cat.# A7231), Vacuum trap for waste collection (e.g., Fisher Cat.# 10-182-50B, 1L size), a vacuum pump capable of 15–20 inches of Hg (e.g., Fisher Cat.# 01-092-29), vacuum tubing, and Miniprep Vacuum Adapters for the Vac-Man® Vacuum Manifold (Cat.# A1331).
- single pipettors capable of dispensing 10–1,000µl

3.A. Preparation of Solutions

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

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

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

Digestion Solution Master Mix Components	Volume per Sample	Volume for N Samples
Nuclei Lysis Solution	200µl	(N + 1) × 200µl
0.5M EDTA (pH 8.0)	50µl	(N + 1) × 50µl
proteinase K, 20mg/ml	20µl	(N + 1) × 20µl
RNase A Solution	5µl	(N + 1) × 5µl
Total Volume	**275µl**	**(N + 1) × 275µl**

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. Carefully seal the Column Wash Solution (CWA) and store at room temperature.

3.B. Preparation of Mouse Tail and Tissue Lysates

1. Cut between 0.5 and 1.2cm length of mouse tail from the tip. Alternatively, weigh **up to 20mg** of tissue sample. A 1.2cm mouse tail clipping usually weighs approximately 20mg. Cut the mouse tail clipping or tissue sample into two equally sized pieces and place them in a 1.5ml microcentrifuge tube.

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

2. Add 275µl of the prepared Digestion Solution Master Mix to each sample tube. Be sure that the sample is completely covered with the Digestion Solution Master Mix. If the mouse tail clipping or tissue sample is not covered by the Digestion Solution Master Mix, cut the tissue into smaller pieces.

3. Incubate the sample tubes overnight (16–18 hours) in a 55°C heat block or water bath. It is not necessary to shake the tubes during incubation.
   Note: Lystes must be warm for processing.

   Optional: After overnight proteinase K digestion, centrifuge samples at 2000 × *g* to pellet any undigested hair or cartilage. Transfer supernatant to a new 1.5ml microcentrifuge tube.

4. Add 250µl of Wizard® SV Lysis Buffer to each sample. Vortex to mix.

5. Process the tissue lysate as soon as possible after the Wizard® SV Lysis Buffer has been added (lysate must remain warm). If the lysates cannot be processed immediately, they may be frozen at –70°C. However, frozen lysates must be thawed and warmed at 55°C for an hour before continuing with the purification.

6. For purification of genomic DNA using a spin protocol with a microcentrifuge, proceed to Section 3.C. For purification of genomic DNA using a Vac-Man® Vacuum Manifold, proceed to Section 3.D.

3.C. Purification of Genomic DNA from Lysates Using a Microcentrifuge

Prepare one Wizard® SV Minicolumn assembly for each lysate. Each minicolumn assembly consists of a Wizard® SV Minicolumn and a Collection Tube. Label the Collection Tube and place the Wizard® SV Minicolumn assembly in a microcentrifuge tube rack.

1. Transfer the entire sample lysate from the 1.5ml microcentrifuge tube to a Wizard® SV Minicolumn assembly.
2. Place the Wizard® SV Minicolumn assembly containing the sample lysate into a microcentrifuge and spin at 13,000 × *g* for 3 minutes to bind the genomic DNA to the Wizard® Minicolumn. If some lysate remains on the column after the initial spin, spin again for 1 minute at 13,000 × *g*.
3. Remove the Wizard® SV Minicolumn from the Minicolumn assembly and discard the liquid in the Collection Tube. Replace the Wizard® SV Minicolumn into the Collection Tube.
4. Verify that ethanol has been added to the Column Wash Solution (CWA) as described in Section 3.A.
5. Add 650µl of Column Wash Solution (CWA) to each Wizard® SV Minicolumn assembly.

   Note: Washing with 650µl in the spin protocol results in DNA of equivalent purity to that obtained by washing with 800µl in the vacuum protocol.
6. Centrifuge at 13,000 × *g* for 1 minute.
7. Discard the liquid in the Collection Tube and replace the Wizard® SV Minicolumn into the empty Collection Tube.
8. Repeat Steps 5–7 three times for a total of four washes of the Wizard® SV Minicolumn.
9. After the last wash, empty the Collection Tube and reassemble the Wizard® SV Minicolumn assembly. Centrifuge at 13,000 × *g* for 2 minutes to dry the binding matrix.
10. Remove the Wizard® SV Minicolumn and place in a new labeled 1.5ml microcentrifuge tube for elution (not provided). Add 250µl of room temperature Nuclease-Free Water to the Wizard® SV Minicolumn. 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.
11. Place the Wizard® SV Minicolumn/elution tube assembly into the centrifuge and spin at 13,000 × *g* for 1 minute.

12. Remove the Wizard® SV Minicolumn/elution tube assembly from the centrifuge. Add another 250µl of Nuclease-Free Water to the Wizard® SV Minicolumn and incubate at room temperature for 2 minutes. Place the Wizard® SV Minicolumn/elution tube assembly into the centrifuge and spin at 13,000 × *g* for 1 minute.

13. Total elution volume will be approximately 500µl.

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

14. Remove the Wizard® SV Minicolumn and discard. Cap the elution tube containing the purified genomic DNA and store at –20 to –70°C.

3.D. Purification of Genomic DNA from Lysates Using a Vac-Man® Vacuum Manifold

For each sample lysate, use one Wizard® SV Minicolumn. Label each SV Minicolumn to maintain sample identity. If the SV Minicolumn becomes clogged during the vacuum procedure, you can switch to the procedure for purification of the DNA by centrifugation (Section 3.C).

1. Attach one Miniprep Vacuum Adapter (Cat.# A1331) with Luer-Lok® fitting to one port of the manifold. Gently press a Wizard® SV Minicolumn into the Miniprep Vacuum Adapter until snugly in place. Ensure that all unused ports of the vacuum manifold are closed. **Note:** Miniprep Vacuum Adapters are reusable.

2. Transfer the entire prepared sample lysate (Section 3.B) to a Wizard® SV Minicolumn.

3. Apply a vacuum until the lysate passes through the Wizard® SV Minicolumn. After the lysate has passed through the column completely, close the one-way Luer-Lok® stopcock of that port.

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

5. Add 800µl of Wash Solution to each Wizard® SV Minicolumn. Apply a vacuum until the Wash Solution has passed through the SV Minicolumn completely. Close each port after the Wash Solution has passed through.

6. Repeat Step 5 three more times for a total of 4 washes of the Wizard® SV Minicolumn.

7. Following the last wash, open each port and continue to pull a vacuum for 4 minutes to dry the binding matrix.

8. Close each port.

9. Turn off the vacuum source and open an unused port to vent the manifold. If all 20 ports are in use, break the vacuum at the source. Make certain that all vacuum pressure is released before performing the next step. It is important to turn off the vacuum at the source to prevent backflow into the columns.

3.D. Purification of Genomic DNA from Lysates Using a Vac-Man® Vacuum Manifold (continued)

10. Remove the Wizard® SV Minicolumn and place in a new 1.5ml labeled microcentrifuge tube (not provided). Add 250µl of room temperature Nuclease-Free Water to the Wizard® SV Minicolumn. 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.

11. Place the Wizard® SV Minicolumn/elution tube assembly into the centrifuge and spin at 13,000 × *g* for 1 minute.

12. Remove the Wizard® SV Minicolumn/elution tube assembly from the centrifuge. Add another 250µl of Nuclease-Free Water to the Wizard® SV Minicolumn and incubate at room temperature for 2 minutes. Place the Minicolumn/elution tube assembly into the centrifuge and spin at 13,000 × *g* for 1 minute.

13. Total elution volume will be approximately 500µl.

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

14. Remove the Wizard® SV Minicolumn and discard. Cap the elution tube containing the purified DNA and store at –20 to –70°C.

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 Column Wash Solution (CWA) prior to beginning the Wizard® SV 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. Carefully seal the Wash Solution and store at room temperature.

4.B. Preparation of Tissue Culture Cell Lysates

Use the following protocol for lysis of adherent cultured cells grown in a 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 1X PBS.

2. Add 150µl of Wizard® SV Lysis Buffer to the washed cells in the tissue culture plate. Mix lysate by pipetting.

3. If the cell lysates will not be used immediately, they can be frozen at –70°C until needed.

4. For purification of genomic DNA from tissue culture cells using a micro-centrifuge, proceed to Section 4.C. For purification of genomic DNA from tissue culture cells using a vacuum, proceed to Section 4.D.

4.C. Purification of Genomic DNA from Tissue Culture Cell Lysates Using a Microcentrifuge

For each lysate to be purified, prepare one Wizard® SV Minicolumn assembly. Each Wizard® SV Minicolumn assembly consists of a Wizard® SV Minicolumn and a Collection Tube. Label the Collection Tube and place the Wizard® SV Minicolumn assembly in a microcentrifuge tube rack.

1. Transfer the entire sample lysate from the tissue culture plate to a Wizard® SV Minicolumn assembly.

2. Place the Wizard® SV Minicolumn/Collection Tube assembly containing the sample lysate into a microcentrifuge and spin at 13,000 × *g* for 3 minutes. If some lysate remains on the column after the initial spin, centrifuge again for 1 minute at 13,000 × *g*.

3. Remove the Wizard® SV Minicolumn from the Minicolumn/Collection Tube assembly and discard the liquid in the Collection Tube. Replace the Wizard® SV Minicolumn into the Collection Tube.

4. Verify that the ethanol has been added to the Column Wash Solution (CWA) as described in Section 4.A.

5. Add 650µl of Column Wash Solution (CWA) to each Minicolumn/Collection Tube assembly.

6. Centrifuge at 13,000 × *g* for 1 minute.

7. Discard the liquid in the Collection Tube and replace the Wizard® SV Minicolumn into the empty Collection Tube.

8. Repeat Steps 5–7 three times for a total of four washes of the Wizard® SV Minicolumn.

9. After the last wash, empty the Collection Tube and reassemble the Wizard® SV Minicolumn/Collection Tube assembly. Centrifuge for 13,000 × *g* for 2 minutes to dry the binding matrix.

   Note: RNA may be co-purified with genomic DNA. To remove copurified RNA, add 2µl of RNase A Solution per 250µl of Nuclease-Free Water prior to elution of genomic DNA from the column. Once eluted, incubate purified genomic DNA at room temperature for 10 minutes. Alternatively, the RNase A Solution (2µl) may be added following elution from the Wizard® SV Minicolumn.

4.C. Purification of Genomic DNA from Tissue Culture Cell Lysates Using a Microcentrifuge (continued)

10. Remove the Wizard® SV Minicolumn and place in a new, labeled 1.5ml microcentrifuge tube (not provided). Add 250µl of room temperature Nuclease-Free Water to the Wizard® SV Minicolumn. 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.

11. Place the Wizard® SV Minicolumn/elution tube assembly into the centrifuge and spin at 13,000 × *g* for 1 minute.

12. Total elution volume will be approximately 250µl.

    Note: Elution volumes of 250µl are recommended for optimal DNA yield. Elution in volumes less than 250µl will concentrate the DNA but will decrease the total DNA yield. For tissue culture cell lysates, additional elution volume dilutes the DNA and does not improve yield.

13. Remove the Wizard® SV Minicolumn and discard. Cap the elution tube containing the purified genomic DNA and store at –20 to –70°C.

4.D. Purification of Genomic DNA from Tissue Culture Cell Lysates Using a Vac-Man® Vacuum Manifold

For each sample lysate, use one Wizard® SV Minicolumn. Label each Minicolumn to maintain sample identity. If the Minicolumn becomes clogged during the vacuum procedure, you can switch to the procedure for purification by centrifugation (Section 4.C).

1. Attach one Miniprep Vacuum Adapter (Cat.# A1331) with Luer-Lok® fitting to one port of the manifold. Gently press a Wizard® SV Minicolumn into the Miniprep Vacuum Adapter until snugly in place. Ensure that all the unused ports of the vacuum manifold are closed.

2. Transfer the entire prepared sample lysate (Section 4.B) to a Wizard® SV Minicolumn.

3. Apply a vacuum until the lysate passes through the Wizard® SV Minicolumn. After the lysate has passed through the column completely, close the one-way Luer-Lok® stopcock of that port.

4. Verify that ethanol has been added to the Column Wash Solution (CWA) as directed in Section 4.A.

5. Add 800µl of Column Wash Solution (CWA) to each Wizard® SV Minicolumn. Apply a vacuum until the Wash Solution has passed through the Minicolumn completely. Close each port after the Wash Solution has passed through the Minicolumn.

6. Repeat Step 5 three times for a total of 4 washes of the Wizard® SV Minicolumn.

7. Following the last wash, open each port and continue to pull a vacuum for 4 minutes to dry the binding matrix. Close each port.

8. Turn off the vacuum source and open an unused port to vent the manifold. If all ports are in use, break the vacuum at the source. Make certain that all vacuum pressure is released before performing the next step. It is important to turn off the vacuum at the source to prevent backflow into the columns.

   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 genomic DNA from the column. Once eluted, incubate purified genomic DNA at room temperature for 10 minutes. Alternatively, the RNase A Solution (2µl) may be added following elution from the Wizard® SV Minicolumn.

9. Remove the Wizard® SV Minicolumn from the vacuum adapter and place into a new 1.5ml labeled microcentrifuge tube (not provided). Add 250µl of room temperature Nuclease-Free Water to the Minicolumn. 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.

10. Place the Wizard® SV Minicolumn/elution tube assembly into the centrifuge and spin at 13,000 × *g* for 1 minute.

11. Total elution volume will be approximately 250µl.

    Note: Elution volumes of 250µl are recommended for optimal DNA yield. Elution in volumes less than 250µl will concentrate the DNA but will decrease the total DNA yield. For tissue culture cell lysates, additional elution volume dilutes the DNA and does not improve yield.

12. Remove the Wizard® SV Minicolumn and discard. Cap the elution tube containing the purified DNA and store at –20 to –70°C.

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 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 that have undergone multiple freeze-thaw cycles may eventually experience DNA degradation. Use fresh tissue samples whenever possible.
	Tissue culture cells are low in genomic DNA. Genomic DNA yield may vary depending on the number of cells used for the isolation. If yields are low, increase the amount of starting material to a maximum of 20mg of tissue or 5×10^6 tissue culture cells.
	Wizard® SV Lysis Buffer not added to tissue lysates or washed cells. Make sure the Wizard® SV Lysis Buffer is added to all sample lysates.
	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 purification.
	Steps not followed correctly or wrong reagents used. The Wizard® SV 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 the purification process. 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.

5. Troubleshooting (continued)

Symptoms	Causes and Comments
RNA contamination	RNase A was not added to the tissue lysate digestion solution. Add 2µl of RNase A Solution to 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 column	Lysate too concentrated or 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 the column.
	Too much tissue sample was used in the lysate preparation. A maximum weight 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 on the column membrane.
	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 at 55°C for 30 minutes and continue with the purification.
	Proteinase K treated mouse tail clipping contains 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 is not easily digested by proteinase K. After proteinase K digestion centrifuge the sample at 2,000 × *g* to pellet undigested sample. Transfer supernatant to a new 1.5ml microcentrifuge tube prior to adding Wizard® SV Lysis Buffer.

5. Troubleshooting (continued)

Symptoms	Causes and Comments
Incomplete digestion of tissue samples	Make sure that Proteinase K was added to the Digestion Solution Master Mix.
	Too much tissue sample was used in the lysate preparation. A **maximum weight of 20mg** mouse tail or animal tissue can be used for lysate preparation.
	Multiple freeze-thaw cycles of proteinase K. Multiple freeze-thaw cycles reduce 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 Minicolumn may be slow.
	Insufficient vacuum pressure. A vacuum pressure >15 inches of mercury is required to use the Wizard® SV Minicolumns.

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 96 Genomic DNA Purification System	1 × 96 preps	A2370
	4 × 96 preps	A2371
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

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