Wizard® SV Genomic DNA Purification System

INSTRUCTIONS FOR USE OF PRODUCTS A2360, A2361 AND A2365.

Quick PROTOCOL

Purification of Genomic DNA from Mouse Tail Clippings or Animal Tissues Using Vacuum

Sample Preparation

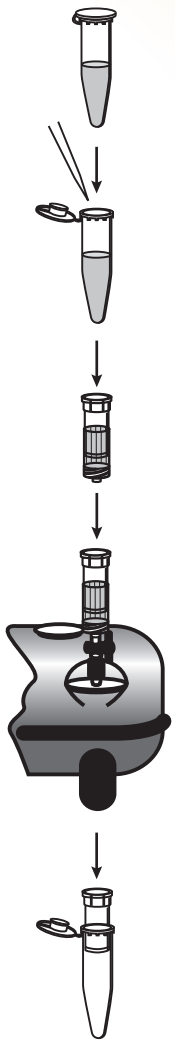
1. Cut a 0.5 to 1.2cm length of mouse tail or weigh up to 20mg of tissue sample. Cut the clipping or tissue sample into two pieces and place them in a 1.5ml microcentrifuge tube.
2. Add 275µl of Digestion Solution Master Mix to each tube.

Digestion Solution Master Mix	Volume per Sample
Nuclei Lysis Solution	200µl
0.5M EDTA (pH 8.0)	50µl
Proteinase K, 20mg/ml	20µl
RNase A Solution, 4mg/ml	5µl
Total Volume	275µl

3. Incubate the sample tubes overnight (16–18 hours) in a 55°C heat block.
4. Add 250µl of Wizard® SV Lysis Buffer to each sample. Vortex.
5. Process the lysate as soon as possible after adding the Lysis Buffer. If frozen at –70°C, lysates must be thawed and heated at 55°C for one hour prior to processing. Lysates must be warm for processing.

Purification of Genomic DNA Using a Vac-Man® Vacuum Manifold

6. For each lysate, use one Wizard® SV Minicolumn. Attach one Miniprep Vacuum Adapter with Luer-Lok® fitting to one port of the manifold. Gently press a minicolumn into the vacuum adapter. Ensure that all unused ports of the vacuum manifold are closed.
7. Transfer the prepared sample lysate to the Wizard® SV Minicolumn.
8. Apply a vacuum until the lysate passes through the minicolumn. After the lysate has passed through the column, close the Luer-Lok® stopcock.
9. Add 800µl of Column Wash Solution (CWA; with 95% ethanol added) to the minicolumn. Apply a vacuum until the solution has passed through the column, then close the port. Repeat this step for a total of 4 washes.
10. Open each port and continue to pull a vacuum for 4 minutes to dry the binding matrix.
11. Close each port. Turn off the vacuum source and break the vacuum.
12. Remove the Wizard® SV Minicolumn and place in a new 1.5ml tube. Add 250µl of room temperature Nuclease-Free Water to the minicolumn. Incubate for 2 minutes at room temperature.
13. Place the minicolumn/tube assembly into the centrifuge and spin at 13,000 x *g* for 1 minute. Repeat Steps 12–13 for a total of 2 elutions (500µl).
14. Remove the minicolumn. Store the purified DNA at –20 to –70°C.



Digest mouse tail clipping or tissue sample in Proteinase K Digestion Solution.

Incubate at 55°C for 16–18 hours.

Add Wizard® SV Lysis Buffer to each sample. Vortex.

Transfer warm lysate to minicolumn.

Apply vacuum until lysate passes through column.

Wash.

Dry matrix with vacuum.

Transfer spin column to microcentrifuge tube. Add Nuclease-Free Water and centrifuge.

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Quick PROTOCOL

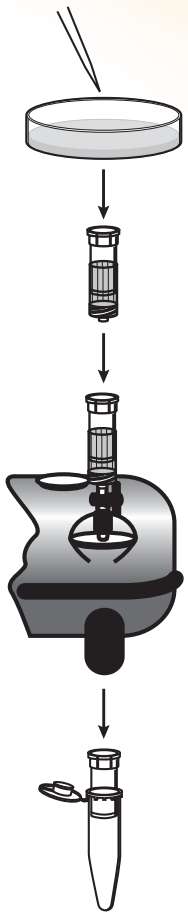
Purification of Genomic DNA from Tissue Culture Cells Using Vacuum

Sample Preparation

1. Use at least 1×10^4 cells to a maximum of 5×10^6 cells. Wash the cells once with 1X PBS.
2. Add 150µl of Wizard® SV Lysis Buffer to the washed cells in a 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.

Purification of Genomic DNA Using a Vac-Man® Vacuum Manifold

4. For each lysate, use one Wizard® SV Minicolumn. Attach one Miniprep Vacuum Adapter with Luer-Lok® fitting to one port of the manifold. Gently press a minicolumn into the vacuum adapter. Ensure that all unused ports of the vacuum manifold are closed.
5. Transfer the prepared sample lysate to the Wizard® SV Minicolumn.
6. Apply a vacuum until the lysate passes through the minicolumn. After the lysate has passed through the column, close the Luer-Lok® stopcock.
7. Add 800µl of Column Wash Solution (CWA; with 95% ethanol added) to the minicolumn. Apply a vacuum until the solution has passed through the column, then close the port. Repeat this step for a total of 4 washes.
8. Open each port and continue to pull a vacuum for 4 minutes to dry the binding matrix.
9. Close each port. Turn off the vacuum source and break the vacuum.
10. Remove the Wizard® SV Minicolumn and place in a new 1.5ml tube. Add 250µl of room temperature Nuclease-Free Water to the minicolumn. Incubate for 2 minutes at room temperature.
11. Place the minicolumn/tube assembly into the centrifuge and spin at 13,000 x *g* for 1 minute. Total elution volume will be approximately 250µl.
12. Remove the minicolumn. Store the purified DNA at –20 to –70°C.



Add Wizard® SV Lysis Buffer to each sample. Pipet to mix.

Transfer lysate to minicolumn.

Apply vacuum until lysate passes through column.

Wash.

Dry matrix with vacuum.

Transfer spin column to microcentrifuge tube. Add Nuclease-Free Water and centrifuge.

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INSTRUCTIONS FOR USE OF PRODUCTS A2360, A2361 AND A2365.

Quick PROTOCOL

Purification of Genomic DNA from Mouse Tail Clippings or Animal Tissues Using a Microcentrifuge

Sample Preparation

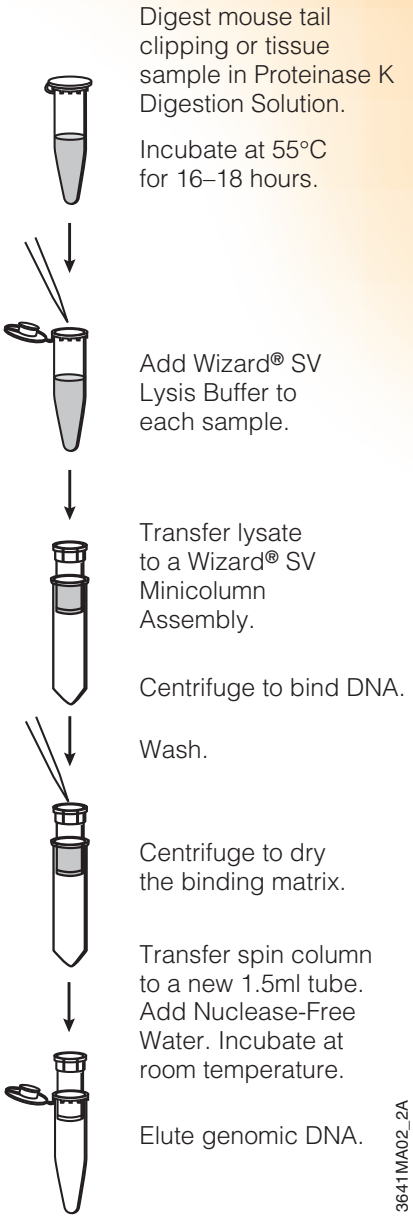
1. Cut a 0.5 to 1.2cm length of mouse tail or weigh up to 20mg of tissue sample. Cut the clipping or tissue sample into two pieces and place them in a 1.5ml microcentrifuge tube.
2. Add 275µl of Digestion Solution Master Mix to each tube.

Digestion Solution Master Mix	Volume per Sample
Nuclei Lysis Solution	200µl
0.5M EDTA (pH 8.0)	50µl
Proteinase K, 20mg/ml	20µl
RNase A Solution, 4mg/ml	5µl
Total Volume	275µl

3. Incubate the sample tubes overnight (16–18 hours) in a 55°C heat block.
4. Add 250µl of Wizard® SV Lysis Buffer to each sample. Vortex.
5. Process the lysate as soon as possible after adding the Lysis Buffer. If frozen at –70°C, lysates should be thawed and heated at 55°C for one hour prior to processing. Lysates must be warm for processing.

Purification of Genomic DNA from Lysate Using a Microcentrifuge

6. Transfer each sample lysate from the 1.5ml tube to a separate Wizard® SV Minicolumn Assembly.
7. Spin the Assembly at 13,000 × *g* for 3 minutes.
8. Remove minicolumn from the Assembly and discard the liquid in the Collection Tube. Replace the minicolumn into the Collection Tube.
9. Add 650µl of Column Wash Solution (CWA; with 95% ethanol added) to each assembly. Centrifuge at 13,000 × *g* for 1 minute. Discard the liquid from the Collection Tube. Repeat this step for a total of 4 washes.
10. Discard the liquid from the Collection Tube and reassemble the minicolumn assembly. Centrifuge for 2 minutes at 13,000 × *g* to dry the binding matrix.
11. Transfer the Wizard® SV Minicolumn to a new 1.5ml tube. Add 250µl of room temperature Nuclease-Free Water. Incubate for 2 minutes at room temperature.
12. Centrifuge the minicolumn/elution tube assembly at 13,000 × *g* for 1 minute. Do not discard the liquid in the elution tube.
13. Add an additional 250µl of Nuclease-Free Water and incubate at room temperature for 2 minutes. Centrifuge the minicolumn/elution tube assembly at 13,000 × *g* for 2 minutes.
14. Remove the minicolumn and store the purified DNA at –20 to –70°C.



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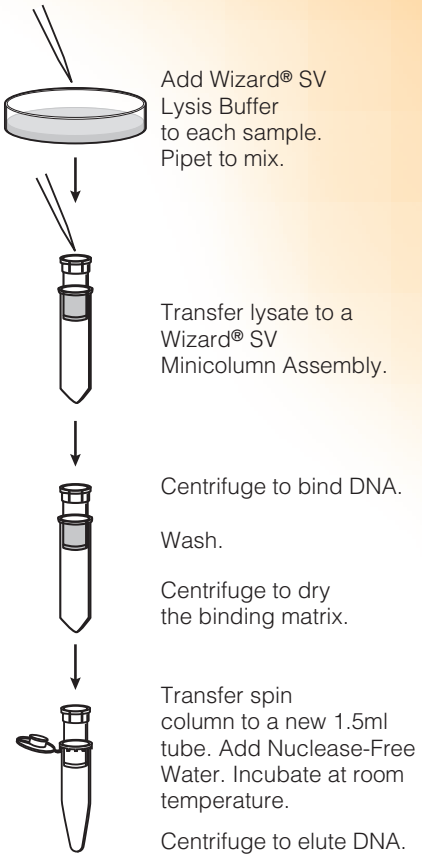
Sample Preparation

1. Use at least 1×10^4 cells to a maximum of 5×10^6 cells. Wash the cells once with 1X PBS.
2. Add 150µl of Wizard® SV Lysis Buffer to the washed cells in a 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.

Purification of Genomic DNA from Tissue Culture Cell Lysate Using a Microcentrifuge

4. Transfer each sample lysate from the tissue culture plate to a separate Wizard® SV Minicolumn Assembly.
5. Spin the Assembly at 13,000 × *g* for 3 minutes.
6. Remove minicolumn from the Assembly and discard the liquid in the Collection Tube. Replace the minicolumn into the Collection Tube.
7. Add 650µl of Column Wash Solution (CWA; with 95% ethanol added) to each assembly. Centrifuge at 13,000 × *g* for 1 minute. Discard the liquid from the Collection Tube. Repeat this step for a total of 4 washes.
8. Discard the liquid from the Collection Tube and reassemble the minicolumn assembly. Centrifuge for 2 minutes at 13,000 × *g* to dry the binding matrix.
9. Transfer the Wizard® SV Minicolumn to a new 1.5ml tube. Add 250µl of room temperature Nuclease-Free Water. Incubate for 2 minutes at room temperature.
10. Centrifuge the minicolumn/elution tube assembly at 13,000 × *g* for 1 minute. Total elution volume will be 250µl.
11. Remove the minicolumn and store the purified DNA at –20 to –70°C.



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