

Wizard® SV 96 Genomic DNA Purification System

INSTRUCTIONS FOR USE OF PRODUCTS A2370 AND A2371.

Quick
PROTOCOL

Purification of Genomic DNA from Mouse Tail Clippings, Animal Tissues and Tissue Culture Cells

Preparation of Mouse Tail Clipping and Tissue Samples

1. Cut a 0.5 to 1.2cm mouse tail clipping or weigh up to 20mg tissue sample. Cut the mouse tail clipping or tissue sample into two pieces and place the pieces into a 96-well, deep-well plate (not provided).

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

2. Add 275µl of the Digestion Solution Master Mix to each sample. Be sure that the sample is covered by the solution. Cover the plate with an adhesive seal.
3. Place the plate in a 55°C water bath and incubate overnight (16–18 hours). Be sure that water does not cover the sample plate. Do not shake.
4. After incubation, dispense 250µl Wizard® SV Lysis Buffer into each well. Lysates must be warm during processing. Mix the contents by pipetting.

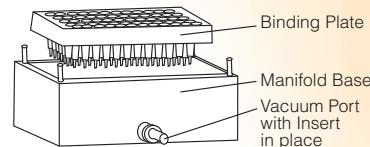
Purification of Genomic DNA from Tail Clippings or Tissue Samples

5. Prepare the vacuum manifold as shown in the figure. Place the Binding Plate in the Manifold Base. Attach the vacuum line to the vacuum port on the Manifold Base.
6. Transfer the tissue lysates to the wells of the Binding Plate. Apply vacuum until all of the lysate has passed through the Binding Plate.
7. Add 1ml of Column Wash Solution (CWA; containing 95% ethanol) to each well.
8. Apply vacuum until the Column Wash Solution (CWA) passes through the Binding Plate. Repeat Steps 7 and 8 for a total of 3 washes.
9. After the wells have emptied continue to apply vacuum for an additional 6 minutes to dry the binding matrix.
10. Turn off the vacuum. Release the vacuum line from the Manifold Base and snap it to the vacuum port in the Vacuum Manifold Collar. Remove the Binding Plate and blot gently to remove 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 Collar on top of the Deep Well Plate as shown.
13. Add 250µl Nuclease-Free Water to each well of the Binding Plate and incubate for 2 minutes at room temperature. (Protocol continued on other side.)

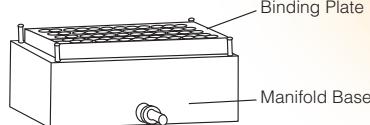
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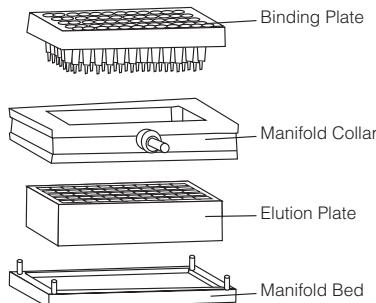
A. Genomic DNA Binding Apparatus



B. Washing Apparatus



C. Elution Apparatus



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Purification of Genomic DNA from Mouse Tail Clippings, Animal Tissues or Tissue Culture Cells (continued)

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 500 μ l.
16. Release the vacuum and remove the Binding Plate. Remove the Manifold Collar, making sure the Deep Well Plate remains positioned in the Manifold Bed. If droplets are present on the top of the wells, gently tap the plate on the bench top. Eluate volumes may vary but are generally 400–450 μ l. Store samples at –20 or –70°C by covering the plate tightly with a plate sealer.

Purification of Genomic DNA from Tissue Culture Cells

1. Wash the cells once with sterile 1X PBS.
2. Add 150 μ l of Wizard® SV Lysis Buffer to the washed cells. Pipet to mix.
3. Prepare the vacuum manifold as shown in the figure. Place the Binding Plate in the Manifold Base. Attach the vacuum line to the vacuum port on the Manifold Base.
4. Transfer the cell lysates to the wells of the Binding Plate. Apply vacuum until all lysate has passed through the wells of the Binding Plate.
5. Add 1ml of Column Wash Solution (CWA; containing 95% ethanol) to each well.
6. Apply vacuum until the Column Wash Solution (CWA) has passed through the Binding Plate. Repeat Steps 5 and 6 for a total of 3 washes.
7. After the wells have emptied, continue to apply vacuum for an additional 6 minutes to dry the binding matrix.
8. Turn off the vacuum. Release the vacuum line from the Manifold Base and snap it to the vacuum port in the Vacuum Manifold Collar. Remove the Binding Plate and blot gently to remove residual ethanol.
9. 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.
10. Position the Binding Plate on top of the Manifold Collar. Place the Collar on top of the Deep Well Plate as shown.
11. Add 250 μ l Nuclease-Free Water to each well of the Binding Plate and incubate for 2 minutes at room temperature.
12. Apply vacuum for 1 minute.
13. Release the vacuum and remove the Binding Plate. Remove the Manifold Collar, making sure the Deep Well Plate remains positioned in the Manifold Bed. If droplets are present on the top of the wells, gently tap the plate on the bench top. Eluate volumes are generally 225 μ l. Store samples at –20 or –70°C by covering the plate tightly with a plate sealer.

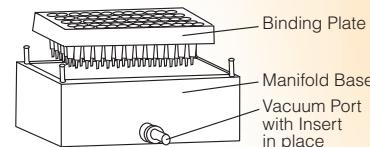
See additional protocol information in Technical Bulletin #TB303, available online at:

www.promega.com/protocols

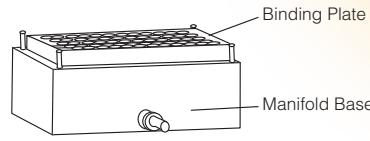
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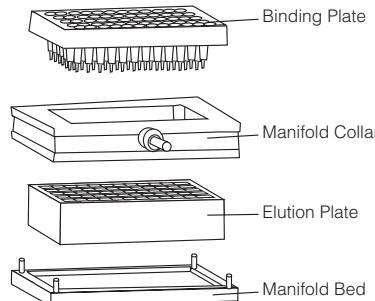
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