Certificate of Analysis

Wizard® Genomic DNA Purification Kit: Trial Size

Cat. No.

Size

A1123

 $10 \times 300 \mu l$ preps



Instructions for use of this product can be found in the Wizard® Genomic DNA Purification Kit Technical Manual #TM050, available online at:

www.promega.com/tbs

Description: The Wizard® Genomic DNA Purification Kit is designed for isolation of DNA from fresh whole blood as well as other tissues and cells (1). High molecular weight genomic DNA can be isolated from human whole blood using this system. Genomic DNA from whole blood collected in EDTA-, heparin- and citrate-anticoagulant tubes all perform equally well in post-purification applications.

DNA purified with this system is suitable for a variety of subsequent applications:

- restriction endonuclease digestion
- membrane hybridization (e.g., Southern and dot/slot blots)
- PCR amplification (2)

For more information about the Wizard® Genomic DNA Purification Kit, refer to Technical Manual #TM050, available online at: www.promega.com/tbs/

Starting materials that can be applied to the Wizard® Genomic DNA Purification Kit:

- mammalian blood
- tissue culture cells
- animal tissue
- plant leaf tissue
- Gram negative bacteria
- Gram positive bacteria
- yeast

This kit consists of enough of the following components to purify genomic DNA based upon the supplied protocol.

Cell Lysis Solution (A793B)

Nuclei Lysis Solution (A794B).

RNase A Solution (A797A): If precipitation has formed, flick the tube to resuspend.

Protein Precipitation Solution (A795B).

DNA Rehydration Solution (A796B).

Storage Temperature: Store the Wizard® Genomic DNA Purification Kit at room temperature (22–25°C). The performance of this product is guaranteed for at least six months from the date of purchase if stored and handled properly.

Quality Control Assays

Each component of the system has been tested and passed its pH, conductivity or RNase activity measurements according to individual quality control specifications.

References

- Miller, S.A., Dykes, D.D. and Polesky, H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucl. Acids Res. 16, 1215.
- Beutler, E., Gelbart, T. and Kuhl, W. (1990) Interference of heparin with the polymerase chain reaction. BioTechniques 9, 166.

Wizard® Genomic DNA Purification Kit, Trial Size

REF A1123



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For In Vitro Research Use Only

Country of Origin: USA

Promega Corporation 2800 Woods Hollow Road Madison, WI 53711-5399 USA





ADA1123 00002585883



Promega Corporation			
2800 Woods Hollow Roa	d		
Madison, WI 53711-539	9 USA		
Telephone	608-274-4330		
Toll Free	800-356-9526		
Fax	608-277-2516		
Internet	www.promega.com		

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Signed by:

R. Wheeler, Quality Assurance

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Usage Information

I. Isolation of Genomic DNA from Whole Blood

Materials to Be Supplied by the User

- sterile 1.5ml microcentrifuge tube (for 20–300µl blood volume)
- . 15ml centrifuge tubes (for 1ml blood volume)
- water bath, 37°C
- · isopropanol, room temperature
- · 70% ethanol, room temperature
- water bath, 65°C (optional; for rapid DNA rehydration)

A. Purification Protocol

The Wizard® Genomic DNA Purification protocol can be scaled for volumes of blood from 20µl to 10ml. The table below indicates the volumes of reagents needed to isolate genomic DNA from 20, 50, 100, 300µl and 1ml of blood.

Blood	Lysis Solutions		Protein Precipitation	y 4/	DNA Rehydration
Sample	Cell	Nuclei	Solution	Isopropanol	Solution
20µІ	60µl	20µl	6.7µl	20μΙ	10µl
50µІ	150µl	50µl	16.5µl	50µl	25μΙ
100μΙ	300µІ	100μΙ	33µІ	100µІ	50µl
300µІ	900μΙ	300µl	100µl	300µl	100µl
1ml	3ml	1ml	330µІ	1ml	150µl

- 1. Add blood to tube containing Cell Lysis Solution and invert to mix.
- 2. Incubate at room temperature for 10 minutes.
- Centrifuge at high speed for 20 seconds.
- 4. Discard supernatant.
- Add Nuclei Lysis Solution and mix by pipetting.
 Optional: Add RNase A Solution (1.5µl for 300µl sample, 5µl for 1ml).
- 6. Add Protein Precipitation Solution and vortex to mix.
- 7. Centrifuge at high speed for 3 minutes.
- Transfer supernatant to a new tube containing the appropriate amount of room temperature isopropanol.
- 9. Gently mix by inversion.
- 10. Centrifuge at high speed for 1 minute.
- 11. Discard supernatant.
- 12. Add room temperature 70% ethanol and gently mix by inversion.
- 13. Centrifuge at high speed for 1 minute.
- 14. Aspirate the ethanol, being careful not to disturb the pellet.
- 15. Air-dry the pellet for 10-15 minutes.
- Add DNA Rehydration Solution to tube and incubate at room temperature for 20 minutes. If DNA is not fully resuspended, increase the incubation time to overnight at 4°C.

II. Isolation of Genomic DNA from Tissue Culture Cells

Materials to Be Supplied by the User

- . 1.5ml microcentrifuge tubes
- · trypsin (for adherent tissue culture cells only)
- PBS
- · water bath, 37°C
- · isopropanol, room temperature
- · 70% ethanol, room temperature
- · water bath, 65°C (optional; for rapid DNA rehydration)

A. Purification Protocol

- 1. Pellet cells by centrifugation at 13,000–16,000 \times g for 10 seconds.
- 2. Wash the cell pellet with PBS, vortex.
- Add 600µl of Nuclei Lysis Solution and 3µl of RNase A Solution and mix. Incubate for 15–30 minutes at 37°C. Cool to room temperature.
- 4. Add 200µl Protein Precipitation Solution and vortex.
- Chill on ice for 5 minutes.
- Centrifuge at 13,000–16,000 × g for 4 minutes.
- Transfer the supernatant to a clean tube containing 600µl of room temperature isopropanol.
- Mix gently by inversion.
- 9. Centrifuge at $13,000-16,000 \times g$ for 1 minute.
- 10. Remove the supernatant and add 600µl of room temperature 70% ethanol. Mix.
- 11. Centrifuge at 13,000-16,000 × g for 1 minute.
- 12. Aspirate the ethanol and air-dry the pellet for 15 minutes.
- Rehydrate the DNA in 100µl of DNA Rehydration solution for 1 hour at 65°C or overnight at room temperature.

Additional protocols are available in the Wizard® Genomic DNA Purification Kit Technical Manual, #TM050. This manual is available upon request from Promega or online at: www.promega.com/tbs/

III. Related Products

DNA Purification Systems

Product	Size	Cat.#
Wizard [®] Genomic DNA Purification Kit	100 × 300µl	A1120
	$500 \times 300 \mu l$	A1125
	100 × 10ml	A1620