Wizard® Genomic DNA Purification Kit

INSTRUCTIONS FOR USE OF PRODUCTS A1120, A1123, A1125 AND A1620.

Quick PROTOCOL

Isolation of Genomic DNA from Whole Blood

Sample Size	Lysis Solution: Cell	Lysis Solution: Nuclei	Protein Precipitation Solution	Isopropanol	DNA Rehydration Solution
300µl	900µl	300µl	100µl	300µl	100µl
1ml	3ml	1ml	330µl	1ml	150µl
3ml	9ml	3ml	1ml	3ml	250µl
10ml	30ml	10ml	3.3ml	10ml	800µl

As little as 20µl can be processed with this system. Please see Technical Manual #TM050, Section 3.C.

Red Blood Cell Lysis

1. Using volumes from the table above, combine the appropriate volumes of Cell Lysis Solution and blood. Mix by inversion.
2. Incubate for 10 minutes at room temperature.
3. Centrifuge:

   ≤300µl sample 13,000–16,000 × *g**; 20 seconds
   1–10ml sample 2,000 × *g*; 10 minutes
4. Discard supernatant. Vortex pellet.

Nuclei Lysis and Protein Precipitation

5. Using volumes from the table above, add Nuclei Lysis Solution and mix by inversion.
6. Add Protein Precipitation Solution; vortex for 20 seconds.
7. Centrifuge:

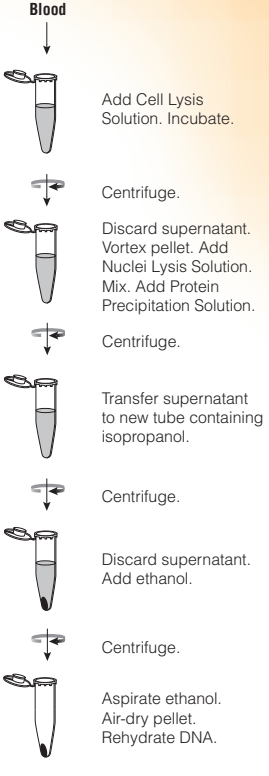
   ≤300µl sample 13,000–16,000 × *g**; 3 minutes
   1–10ml sample 2,000 × *g*; 10 minutes

DNA Precipitation and Rehydration

8. Transfer supernatant to a new tube contaning isopropanol (using volumes from table above). Mix.
9. Centrifuge:

   ≤300µl sample 13,000–16,000 × *g**; 1 minute
   1–10ml sample 2,000 × *g*; 1 minute
10. Discard supernatant. Add 70% ethanol (same volume as isopropanol).
11. Centrifuge as in Step 9.
12. Aspirate the ethanol and air-dry the pellet (10–15 minutes).
13. Rehydrate the DNA in the appropriate volume of DNA Rehydration Solution for 1 hour at 65°C or overnight at 4°C.

*****Maximum speed on a microcentrifuge.



Additional protocol information is available in Technical Manual #TM050, available online at: **www.promega.com**

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Quick PROTOCOL

Isolation of Genomic DNA from Animal Tissue and Tissue Culture Cells

Prepare Tissues

Tissue Culture Cells: Centrifuge at 13,000–16,000 × *g** for 10 seconds. Wash the cell pellet with PBS, vortex and then add 600µl of Nuclei Lysis Solution and mix by pipetting.

Animal Tissue: Add 10–20mg of fresh or thawed tissue to 600µl of chilled Nuclei Lysis Solution and homogenize for 10 seconds. Alternatively, use 10–20mg of ground tissue. Incubate at 65°C for 15–30 minutes.

Mouse Tail: Add 600µl of chilled EDTA/Nuclei Lysis Solution to 0.5–1cm of fresh or thawed mouse tail. Add 17.5µl of 20mg/ml Proteinase K and incubate overnight at 55°C with gentle shaking.

Lysis and Protein Precipitation

1. Add 3µl of RNase Solution to the cell or animal tissue nuclei lysate and mix. Incubate for 15–30 minutes at 37°C. Cool to room temperature.
2. Add 200µl of Protein Precipitation Solution. Vortex and chill on ice for 5 minutes.
3. Centrifuge at 13,000–16,000 × *g** for 4 minutes.

DNA Precipitation and Rehydration

4. Transfer supernatant to a fresh tube containing 600µl of room temperature isopropanol.
5. Mix gently by inversion.
6. Centrifuge at 13,000–16,000 × *g** for 1 minute.
7. Remove supernatant and add 600µl of room temperature 70% ethanol. Mix.
8. Centrifuge as in Step 6.
9. Aspirate the ethanol and air-dry the pellet for 15 minutes.
10. Rehydrate the DNA in 100µl of DNA Rehydration Solution for 1 hour at 65°C or overnight at 4°C.

*Maximum speed on a microcentrifuge.

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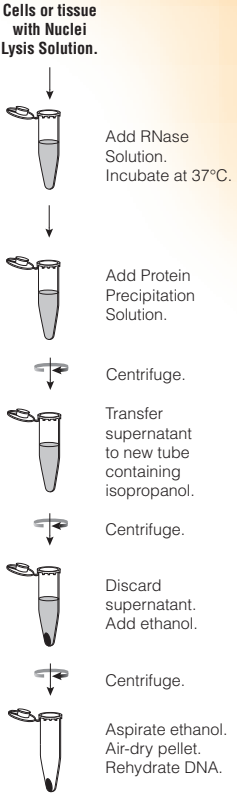

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Quick PROTOCOL

Isolation of Genomic DNA from Gram Positive and Gram Negative Bacteria

Pellet Cells

Centrifuge 1ml of overnight culture for 2 minutes at 13,000–16,000 × *g**.
Discard the supernatant.

A. For Gram Positive Bacteria

1. Suspend cells in 480µl 50mM EDTA.
2 Add lytic enzyme(s) (120µl) [lysozyme and/or lysostaphin].
3 Incubate at 37°C for 30–60 minutes.
4. Centrifuge for 2 minutes at 13,000–16,000 × *g** and remove supernatant.
5. Go to Step 1, **Lyse Cells** (below).

B. For Gram Negative Bacteria

Go to Step 1, **Lyse Cells** (below).

Lyse Cells

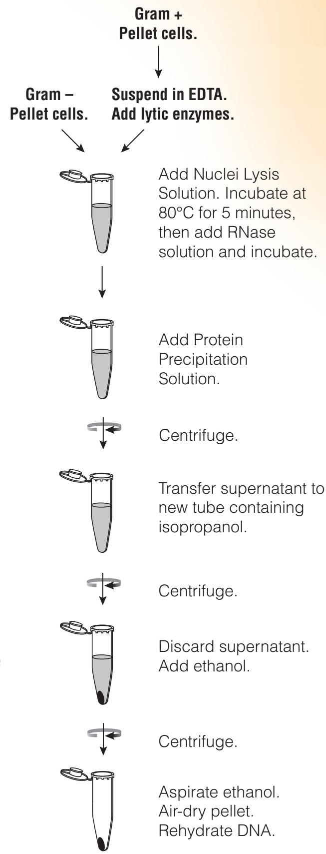
1. Add 600µl Nuclei Lysis Solution. Pipet gently to mix.
2. Incubate for 5 minutes at 80°C, then cool to room temperature.
3. Add 3µl of RNase Solution. Mix, incubate at 37°C for 15–60 minutes, then cool to room temperature.

Protein Precipitation

4. Add 200µl of Protein Precipitation Solution. Vortex.
5. Incubate on ice for 5 minutes.
6. Centrifuge at 13,000–16,000 × *g** for 3 minutes.

DNA Precipitation and Rehydration

7. Transfer the supernatant to a clean tube containing 600µl of room temperature isopropanol. Mix.
8. Centrifuge as in "Pellet Cells" above, and decant the supernatant.
9. Add 600µl of room temperature 70% ethanol. Mix.
10. Centrifuge for 2 minutes at 13,000–16,000 × *g**.
11. Aspirate the ethanol and air-dry the pellet for 10–15 minutes.
12. Rehydrate the DNA pellet in 100µl of Rehydration Solution for 1 hour at 65°C or overnight at 4°C.



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Quick PROTOCOL

Isolation of Genomic DNA from Yeast Cultures or Plant Tissue

Prepare Yeast Lysate

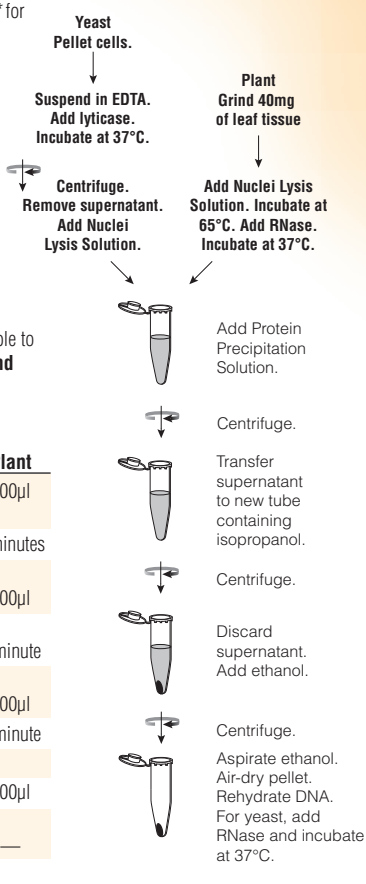
1. Pellet cells from 1ml of culture by centrifugation at 13,000–16,000 × *g** for 2 minutes.
2. Suspend the cell pellet in 293µl of 50mM EDTA.
3. Add 7.5µl of 75 units/µl lyticase and mix gently.
4. Incubate for 30–60 minutes at 37°C. Cool to room temperature.
5. Centrifuge as in Step 1. Discard the supernatant.
6. Add 300µl of Nuclei Lysis Solution. Proceed to **Protein Precipitation and DNA Rehydration,** Step 1 (below).

Prepare Plant Lysate

1. Grind approximately 40mg of leaf tissue in liquid nitrogen.
2. Add 600µl of Nuclei Lysis Solution. Incubate at 65°C for 15 minutes.
3. Add 3µl of RNase Solution. Incubate at 37°C for 15 minutes. Cool sample to room temperature for 5 minutes. Proceed to **Protein Precipitation and DNA Rehydration,** Step 1 (below).

Protein Precipitation and DNA Rehydration

	Yeast	Plant
1. Add Protein Precipitation Solution. Vortex. **For yeast only:** Incubate 5 minutes on ice.	100µl	200µl
2. Centrifuge at 13,000–16,000 × *g**.	3 minutes	3 minutes
3. Transfer supernatant to clean tube containing room temperature isopropanol.	300µl	600µl
4. Mix by inversion and centrifuge at 13,000–16,000 × *g**.	2 minutes	1 minute
5. Decant supernatant and add room temperature 70% ethanol.	300µl	600µl
6. Centrifuge at 13,000–16,000 × *g**.	2 minutes	1 minute
7. Aspirate the ethanol and air-dry the pellet.		
8. Add DNA Rehydration Solution.	50µl	100µl
9. **For yeast only:** Add RNase. Incubate at 37°C for 15 minutes.	1.5µl	—
10. Rehydrate at 65°C for 1 hour or overnight at 4°C.		



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