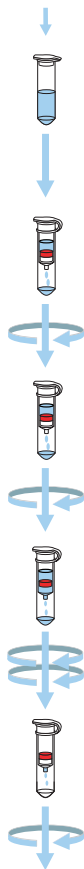


GENELUTE MAMMALIAN GENOMIC DNA KIT

Starting Material



Pure Mammalian Genomic DNA

1 Release DNA from cultured cells, tissues (including rodent tails), fresh whole blood or white blood cells

A. Cultured cells

- Pellet up to 5×10^6 cells. Discard medium.
- Resuspend cells in 200 μ l Resuspension Solution.
- Optional: Add 20 μ l RNase. Mix & incubate at RT, 2 min.*
- Add 20 μ l Proteinase K & 200 μ l Lysis Solution to cell suspension. Vortex or pipet to mix.
- Incubate at 70 °C, 10 min. Proceed to section 2.

B. Mammalian Tissues

- Mince up to 25 mg tissue on ice. Transfer to 1.5-2 ml microcentrifuge tube.
- Add 180 μ l Lysis Solution for Tissue & 20 μ l Proteinase K. Vortex or invert to mix.
- Incubate at 55 °C until fully digested (2-4 h)
- Optional: Add 20 μ l RNase. Mix & incubate at RT, 2 min.*
- Add 200 μ l Lysis Solution. Vortex or pipet to mix. Incubate at 70 °C, 10 min. Proceed to section 2.

C. Rodent tails

- Cut two (mouse) or one (rat) 0.5-0.6 cm pieces from tip of tail on ice. Transfer to 1.5-2 ml microcentrifuge tube.
- Add 180 μ l Lysis Solution for Tissue & 20 μ l Proteinase K. Mix by vortexing. Ensure that tail is fully submerged.
- Incubate at 55 °C until fully digested (3-6 h). Vortex briefly.
- Optional: Add 20 μ l RNase. Mix & incubate at RT, 2 min.*
- Add 200 μ l Lysis Solution; do not mix. Immediately proceed to section 2.

D. Fresh whole blood*

- Collect whole blood in an anticoagulant tube & equilibrate to RT.
- Place 20 μ l of Proteinase K in a 1.5 ml microcentrifuge tube. Add up to 200 μ l of whole blood (or blood + Resuspension Solution to 200 μ l).
- Optional: Add 20 μ l RNase. Mix & incubate at RT, 2 min.*
- Add 200 μ l of Lysis Solution. Vortex or pipet to mix.
- Incubate at 55 °C, 10 min. Proceed to section 2.

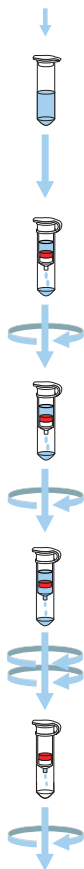
*Also available: GenElute™ Blood Genomic DNA Kit (NA2000, NA2010, NA2020)

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GENELUTE MAMMALIAN GENOMIC DNA KIT

Starting Material



Pure Mammalian Genomic DNA

1 Release DNA from cultured cells, tissues (including rodent tails), fresh whole blood or white blood cells (continued)

E. White blood cells (WBC)

- Prepare WBCs from 500 μl of whole blood/prep. See Appendix in *Technical Bulletin for an Ammonium Chloride Lysis procedure*.
- Resuspend pellet thoroughly in 200 μl of Resuspension Solution. Add 20 μl of Proteinase K & vortex briefly.
- Optional: Add 20 μl RNase. Mix & incubate at RT, 2 min.
- Add 200 μl of Lysis Solution. Vortex or pipet to mix.
- Incubate at 55 $^{\circ}\text{C}$, 10 min. Proceed to section 2.

2 Prepare column

- Add 500 μl Column Preparation Solution to binding column.
- Spin at $\geq 12,000 \times g$, 1 min. Discard flow-through.

3 Bind DNA to column

- Add 200 μl ethanol to lysed cells or tissue. Vortex or invert to mix.
- Transfer to binding column. Spin $\geq 6,500 \times g$, 1 min.

4 Wash to remove contaminants

- Transfer column to new collection tube. Add 500 μl Wash Solution to column. Spin at $\geq 6,500 \times g$, 1 min.
Note: Ethanol must be added to Wash Solution concentrate before first use.
- Transfer column to new collection tube. Add second 500 μl Wash Solution to column.
- Spin at $\geq 12,000 \times g$ for 3 min. to dry column.

5 Elute purified DNA

- Transfer column to new collection tube.
- Add 200 μl Elution Solution. Spin at $\geq 6,500 \times g$, 1 min.
- Optional: Repeat elution in same or new tube.

