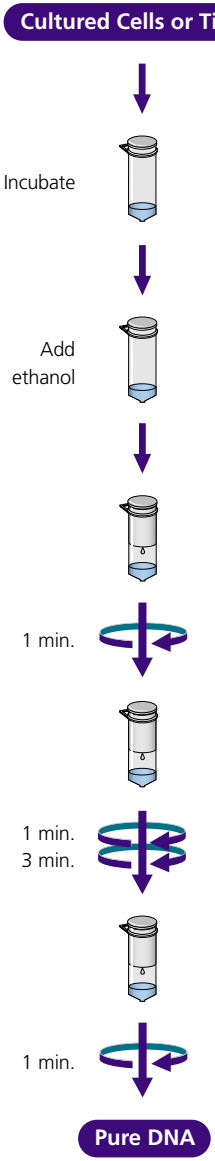
GENELUTE MAMMALIAN GENOMIC DNA KIT



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

A. *Cultured cells*

- ❑ Pellet up to 5 x 10^6 cells. Discard medium.
- ❑ Resuspend cells in 200 µl resuspension solution.
- ❑ *Optional: Add 20 µl RNase. Mix & incubate at RT, 2 min.*
- ❑ Add 20 µl Proteinase K & 200 µl 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 µl lysis solution for tissue & 20 µl Proteinase K. Vortex or invert to mix.
- ❑ Incubate at 55 °C until fully digested (2-4 h)
- ❑ *Optional: Add 20 µl RNase. Mix & incubate at RT, 2 min.*
- ❑ Add 200 µl 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 µl Lysis Solution for Tissue & 20 µl 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 µl RNase. Mix & incubate at RT, 2 min.*
- ❑ Add 200 µl 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 µl of Proteinase K in a 1.5 ml microcentrifuge tube. Add up to 200 µl of whole blood (or blood + Resuspension Solution to 200 µl).
- ❑ *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°C, 10 min. Proceed to section 2.

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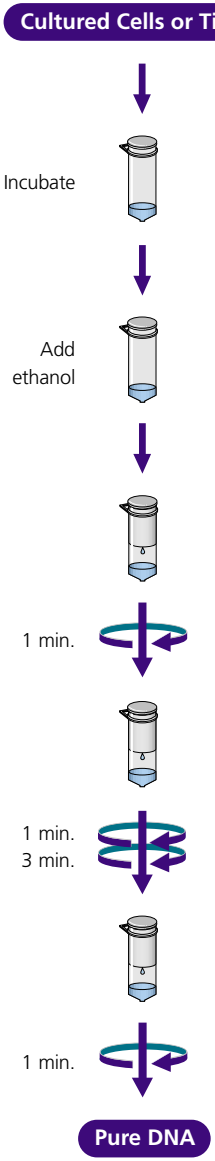

1 Release DNA from cultured cells, tissues (including rodent tails), fresh whole blood or white blood cells *(continued)*

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°C, 10 min. Proceed to section 2.

2 Bind DNA to column

- ❑ Add 200 µl ethanol. Vortex or invert to mix.
- ❑ Transfer to binding column. *Spin ≥ 6,500 x g, 1 min.*

3 Wash to remove contaminants

- ❑ Transfer column to new collection tube. Add 500 µl Wash Solution to column. *Spin at ≥ 6,500 x 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 ≥ 12,000 x g for 3 min. to dry column.*

4 Elute purified DNA

- ❑ Transfer column to new collection tube.
- ❑ Add 200 µl Elution Solution. *Spin at ≥ 6,500 x g, 1 min.*
- ❑ *Optional: Repeat elution in same or new tube.*

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