

DNeasy[®] Blood & Tissue Kit

The DNeasy Blood & Tissue Kit (cat. nos. 69504 and 69506) can be stored at room temperature (15–25°C) for up to 1 year if not otherwise stated on label.

Further information

- *DNeasy Blood & Tissue Handbook*: www.qiagen.com/HB-2061
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: support.qiagen.com

Notes before starting

- Perform all centrifugation steps at room temperature (15–25°C).
 - Redissolve any precipitates in Buffer AL and Buffer ATL.
 - Add ethanol to Buffer AW1 and Buffer AW2 concentrates.
 - Equilibrate frozen tissue or cell pellets to room temperature.
 - Preheat an incubator to 56°C.
 - Refer to the handbook for pretreatment of fixed tissue, insect, bacterial or other material.
- 1a. **Tissue**: Cut tissue (≤ 10 mg spleen or ≤ 25 mg other tissue) into small pieces, and place in a 1.5 ml microcentrifuge tube. For rodent tails, use 1 (rat) or 2 (mouse) 0.4–0.6 cm lengths of tail. Add 180 μ l Buffer ATL. Add 20 μ l proteinase K, mix by vortexing and incubate at 56°C until completely lysed. Vortex occasionally during incubation. Vortex 15 s directly before proceeding to step 2.
 - 1b. **Nonnucleated blood**: Pipet 20 μ l proteinase K into a 1.5 ml or 2 ml microcentrifuge tube. Add 50–100 μ l anticoagulant-treated blood. Adjust volume to 220 μ l with PBS. Proceed to step 2.

- 1c. **Nucleated blood:** Pipet 20 μ l proteinase K into a 1.5 ml or 2 ml microcentrifuge tube. Add 5–10 μ l anticoagulant-treated blood. Adjust volume to 220 μ l with PBS. Proceed to step 2.
- 1d. **Cultured cells:** Centrifuge a maximum of 5×10^6 cells for 5 min at $300 \times g$ (190 rpm). Resuspend in 200 μ l PBS. Add 20 μ l proteinase K. Proceed to step 2.
2. Add 200 μ l Buffer AL. Mix thoroughly by vortexing. Incubate blood samples at 56°C for 10 min.
3. Add 200 μ l ethanol (96–100%). Mix thoroughly by vortexing.
4. Pipet the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuge at $\geq 6000 \times g$ (8000 rpm) for 1 min. Discard the flow-through and collection tube.
5. Place the spin column in a new 2 ml collection tube. Add 500 μ l Buffer AW1. Centrifuge for 1 min at $\geq 6000 \times g$. Discard the flow-through and collection tube.
6. Place the spin column in a new 2 ml collection tube, add 500 μ l Buffer AW2 and centrifuge for 3 min at $20,000 \times g$ (14,000 rpm). Discard the flow-through and collection tube.
7. Transfer the spin column to a new 1.5 ml or 2 ml microcentrifuge tube.
8. Elute the DNA by adding 200 μ l Buffer AE to the center of the spin column membrane. Incubate for 1 min at room temperature ($15\text{--}25^\circ\text{C}$). Centrifuge for 1 min at $\geq 6000 \times g$.
9. **Optional:** Repeat step 8 for increased DNA yield.



Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.

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