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.

For more information, please refer to the *DNeasy Blood & Tissue Handbook*, which can be found at www.qiagen.com/handbooks.

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

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 µl Buffer ATL. Add 20 µl 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 µl proteinase K into a 1.5 ml or 2 ml microcentrifuge tube. Add 50–100 µl anticoagulant-treated blood. Adjust volume to 220 µl with PBS. Proceed to step 2.

1c. **Nucleated blood**: Pipet 20 µl proteinase K into a 1.5 ml or 2 ml microcentrifuge tube. Add 5–10 µl anticoagulant-treated blood. Adjust volume to 220 µl with PBS. Proceed to step 2.

For Material Safety Data Sheets, see www.qiagen.com/safety.

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1d. **Cultured cells**: Centrifuge a maximum of 5 x 10^6 cells for 5 min at 300 x *g* (190 rpm). Resuspend in 200 µl PBS. Add 20 µl proteinase K. Proceed to step 2.

2. Add 200 µl Buffer AL. Mix thoroughly by vortexing. Incubate blood samples at 56°C for 10 min.

3. Add 200 µl 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 ≥ 6000 x *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 µl Buffer AW1. Centrifuge for 1 min at ≥6000 x *g*. Discard the flow-through and collection tube.

6. Place the spin column in a new 2 ml collection tube, add 500 µl Buffer AW2, and centrifuge for 3 min at 20,000 x *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 µl Buffer AE to the center of the spin column membrane. Incubate for 1 min at room temperature (15–25°C). Centrifuge for 1 min at ≥6000 x *g*.

9. **Optional**: Repeat step 8 for increased DNA yield.

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

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