

## DNeasy<sup>®</sup> 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](http://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](http://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 ( $\leq 10$  mg spleen or  $\leq 25$  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  $\mu$ l Buffer ATL. Add 20  $\mu$ 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  $\mu$ l proteinase K into a 1.5 ml or 2 ml microcentrifuge tube. Add 50–100  $\mu$ l anticoagulant-treated blood. Adjust volume to 220  $\mu$ l with PBS. Proceed to step 2.
- 1c. **Nucleated blood:** Pipet 20  $\mu$ l proteinase K into a 1.5 ml or 2 ml microcentrifuge tube. Add 5–10  $\mu$ l anticoagulant-treated blood. Adjust volume to 220  $\mu$ l with PBS. Proceed to step 2.

January 2011



- 1d. **Cultured cells:** Centrifuge a maximum of  $5 \times 10^6$  cells for 5 min at  $300 \times g$  (190 rpm). Resuspend in 200  $\mu\text{l}$  PBS. Add 20  $\mu\text{l}$  proteinase K. Proceed to step 2.
2. Add 200  $\mu\text{l}$  Buffer AL. Mix thoroughly by vortexing. Incubate blood samples at  $56^\circ\text{C}$  for 10 min.
3. Add 200  $\mu\text{l}$  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  $\mu\text{l}$  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  $\mu\text{l}$  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  $\mu\text{l}$  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.

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

Trademarks: QIAGEN®, DNeasy® (QIAGEN Group). 1066955 01/2011  
© 2011 QIAGEN, all rights reserved.

