

# DNeasy<sup>®</sup> 96 Blood & Tissue Kit

The DNeasy 96 Blood & Tissue Kit (cat. nos. 69581 and 69582) 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](http://www.qiagen.com/HB-2061)
- Safety Data Sheets: [www.qiagen.com/safety](http://www.qiagen.com/safety)
- Technical assistance: [support.qiagen.com](http://support.qiagen.com)

## Notes before starting

- Perform all centrifugation steps at room temperature.
- If necessary, redissolve any precipitates in Buffer AL and Buffer ATL.
- Add ethanol to Buffer AW1 and Buffer AW2 concentrates.
- If using tissue, add ethanol to Buffer AL before use.
- If using animal blood, refer to the handbook.
- Equilibrate frozen tissue samples to room temperature.
- Preheat an incubator to 56°C.

## Procedure

1. Cut  $\leq 20$  mg tissue into small pieces, and place into a collection microtube. For rodent tails, use 1 (rat) or 2 (mouse) 0.4–0.6 cm lengths of tail. Use a 96-Well-Plate Register for sample position.
2. Prepare a working solution containing 20  $\mu$ L proteinase K stock solution and 180  $\mu$ L Buffer ATL per sample, and mix by vortexing. Immediately pipet 200  $\mu$ L working solution into each collection microtube. Tightly seal the microtubes using the caps provided.
3. Place the clear cover over each rack, and mix by inverting. Centrifuge to collect any solution from the caps. The samples must be completely submerged in the proteinase K–Buffer ATL solution after centrifugation.
4. Incubate at 56°C overnight or until the samples are completely lysed. Place a weight on top of the caps during the incubation. Mix occasionally during incubation to disperse the sample.

5. Ensure that the microtubes are properly sealed. Cover the racks and vigorously shake up and down for 15 s. Centrifuge to collect any solution from the caps. Ensure that samples are completely lysed to avoid clogging wells of the DNeasy 96 plate.
6. Carefully remove the caps and add 410  $\mu\text{L}$  Buffer AL–ethanol mixture to each sample, and tightly reseal using new caps.
7. Place a clear cover over each rack and shake the racks vigorously up and down for 15 s. Centrifuge to collect any solution from the caps.
8. Place two DNeasy 96 plates on top of S-Blocks. Mark the DNeasy 96 plates for later sample identification.
9. Carefully remove microtube caps and transfer the lysate (maximum 900  $\mu\text{L}$ ) of each sample to each well of the DNeasy 96 plates.
10. Seal each plate with an AirPore Tape Sheet. Centrifuge for 10 min at  $5788 \times g$  (6000 rpm).
11. Remove the tape. Add 500  $\mu\text{L}$  Buffer AW1 to each sample.
12. Seal with a new AirPore Tape Sheet. Centrifuge for 5 min at  $5788 \times g$ .
13. Add 500  $\mu\text{L}$  Buffer AW2 to each sample.
14. Centrifuge for 15 min at  $5788 \times g$  (do not seal the plate with tape).
15. Place each DNeasy 96 plate on a new rack of Elution Microtubes RS.
16. Add 200  $\mu\text{L}$  Buffer AE to each sample, and seal with new AirPore Tape Sheets. Incubate for 1 min at room temperature. Centrifuge for 2 min at  $5788 \times g$ .  
**Optional:** Repeat this step for increased DNA yields.
17. Seal the Elution Microtubes RS with new caps to store the eluted DNA.

## Document Revision History

Date	Changes
04/2016	Initial release
10/2022	Corrected g-force value. Applied new brand template.



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